EFFECT OF FEEDING VARIOUS DRIED DISTILLERS GRAINS PLUS SOLUBLES ON BEEF MEAT QUALITY

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ABSTRACT

Two studies were designed to investigate the effect of feeding crossbred beef steers a standard barley-based control diet or substituting wheat, corn, or wheat/corn (40% dry matter basis) dried distillers grains plus solubles (DDGS) on the resulting meat quality. The first study investigated the effect of diet on the quality of the *longissimus* muscle. It was observed that diet did not have an effect on meat composition, pH, drip loss or shear values; however, diet did have an effect on colour attributes, with steaks obtained from steers fed a DDGS diet losing redness faster over time and having a less desirable retail appearance than steaks obtained from steers fed the control diet ($P<0.001$). Differences ($P<0.05$) were also observed in both the subcutaneous and intramuscular fatty acid profiles, with steers fed corn DDGS having elevated levels of *trans*-monounsaturated fatty acids, and steers fed a DDGS diet having elevated levels of branched-chain fatty acids and conjugated linoleic acid. The fatty acid composition of cattle fed a DDGS diet also had higher ($P<0.05$) concentrations of polyunsaturated fatty acids, which resulted in a less desirable n-6 to n-3 ratio. Despite the fact that fat obtained from steers fed a DDGS diet had elevated levels of total unsaturated fatty acids when compared to steers fed the control diet, no differences were observed in regards to the oxidative stability of raw or cooked meat as determined by thiobarbituric acid reactive substances.

The second study investigated the effect of diet on the quality of raw and pre-cooked *semimembranosus* roasts injected with a salt/phosphate brine. No dietary effects were observed in meat quality, processing attributes, or shear values of the non-injected SM roasts, while roasts prepared from the *semimembranosus* of animals fed corn DDGS had the lowest brine pickup and the highest shear values. Raw non-injected meat from steers fed a DDGS diet was less oxidatively stable than meat obtained from steers fed a control diet. Oxidative changes due to diet were mitigated in the cooked injected roasts due to the chelating effect of phosphate, which improved overall oxidative stability. Similarly, oxidation levels in the pre-cooked SM roasts remained below levels where rancidity is normally detected over 56 days of refrigerated storage at 4°C. Overall, replacing barley grain with 40% wheat, corn, or wheat/corn DDGS did not have a profound impact on meat quality attributes of raw or cooked meat.

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1.0 INTRODUCTION

Considerable interest exists in the use of by-products of the distilling industry as feed ingredients in livestock rations. In recent years, the growth of the ethanol industry has resulted in large increases in the supply and use of dried distillers grains plus solubles (DDGS) in livestock rations. During the production of ethanol, the starch is removed through the fermentation of the feedstock by yeast, resulting in the concentration of the protein and fat components in the remaining residue (Gibb et al., 2008). Ethanol is then removed from the fermented slurry through distillation, with the resulting mash being centrifuged to separate the coarse grains from the solubles (Gibb et al., 2008). The solubles can then be condensed through evaporation, forming a syrup of approximately 30% dry matter, while the coarse grains can be fed directly to livestock or dehydrated to produce dried distillers grains (Gibb et al., 2008). However, if the condensed solubles are added back to the distillers grains before dehydration, the resulting product is known as dried distillers grains plus solubles (Gibb et al., 2008).

Several researchers have studied the effect of feeding corn (Ham et al., 1994; Benson et al., 2005; Klopfenstein et al., 2008; Buckner et al., 2008; Eun et al., 2009) and/or wheat DDGS (Beliveau and McKinnon, 2008; McKinnon and Walker, 2008; Gibb et al., 2008; Walter et al., 2010) on the performance and carcass characteristics of feedlot steers. Several researchers have also evaluated the effect of feeding corn (Roeber et al., 2005; Gill et al., 2008; Leupp et al., 2009; Koger et al., 2010; Kinman et al., 2011) DDGS on meat quality; however, very little research has been conducted evaluating the effect of feeding wheat DDGS (Aldai et al., 2010b) on meat quality. Likewise, no research has been conducted evaluating the effect of feeding wheat/corn DDGS on meat quality. Examining the effect of feeding a wheat/corn blend DDGS is important because, depending on market conditions, wheat and/or corn DDGS may be available. Because the availability of DDGS may change with the economy, it is important to understand the effect of feeding DDGS derived from wheat and corn on

meat quality. Therefore, further research is required to evaluate the effect of feeding wheat DDGS and a blend of wheat and corn DDGS on meat quality.

Although the effect of DDGS diet on meat quality has been extensively studied, much of this work has focused on the meat's composition, colour stability, fatty acid composition, tenderness, and sensory characteristics. In contrast, very little research has been conducted to evaluate the effect of feeding corn DDGS on the oxidative stability (Koger et al., 2010) of meat, while no research has evaluated the effect of feeding wheat DDGS on the oxidative stability of meat. Oxidation of the lipid components affects fatty acids, particularly polyunsaturated fatty acids (Gill et al., 2008) due to a higher degree of unsaturation. It has been well documented that feeding cattle DDGS results in an increase in polyunsaturated fatty acids (Gill et al., 2008; Aldai et al., 2010a; Koger et al., 2010; Kinman et al., 2011). Because so little research has been conducted evaluating the oxidative stability of meat derived from cattle fed DDGS, it is hypothesized that feeding cattle DDGS will negatively impact the oxidative stability of meat due to the increase in polyunsaturated fatty acids, leading to poor colour stability and the development of off-flavours. Hence, further research is required to investigate the oxidative stability of meat derived from cattle fed DDGS.

While several researchers have evaluated the effect of feeding DDGS on meat quality, all of this work has focussed on the quality of the *longissimus* muscle. To date, no research has been conducted to evaluate the effect of DDGS diet on other muscles, such as the *semimembranosus*. Furthermore, the market for convenience products is expanding so in order to develop markets for value-added beef products, such as pre-cooked roasts, it is important to understand how diet and storage conditions will affect both the processing conditions of roast beef and the finished product. To date, no research has been conducted evaluating the effect of feeding DDGS on the quality of *semimembranosus* muscle and the production of value-added meat products. This is important because oxidation challenges may be encountered with fully cooked meat products and oxidation events may be exacerbated with the increase in unsaturated fatty acids in fat that has been reported following DDGS feeding. Therefore, further research is required to investigate the impact DDGS diet has on the production of value-added meat products.

Study 1

Hypothesis: DDGS diet will impact the fatty acid profile of the meat, resulting in a loss of oxidative and colour stability, but it will not impact the meat's composition, cooking characteristics, or tenderness values.

Objectives: To examine the effect of feeding crossbred beef steers various DDGS diets on the oxidative stability of raw and cooked ground beef, colour stability, palatability, fatty acid profile, and textural properties of the *longissimus* muscle.

Study 2

Hypothesis: DDGS diet will not impact processing characteristics or tenderness of meat, while brine injection will mitigate any dietary oxidative differences and improve tenderness.

Objectives: To examine the effect of feeding crossbred beef steers various DDGS diets on processing characteristics, textural properties, water-holding capacity, and oxidative stability of pre-cooked roasts prepared from the *semimembranosus* muscle.

2.0 LITERATURE REVIEW

2.1 Distillers Grain

In an effort to lower our dependence on fossil fuels, availability of a new livestock feed ingredient, distillers grains plus solubles, a by-product of ethanol production, has been increasing due to increased ethanol production. With rising fuel prices and government incentives, there are now more than 1.5 billion litres of fuel ethanol produced in Canada each year (Canadian Renewable Fuels Association, 2007), with one-third of this amount arising from the fermentation of wheat (Gibb et al., 2008). This livestock feed is produced after the starch fraction of the grain, which accounts for approximately 70% of the weight of wheat (Gibb et al., 2008), has been fermented by yeast to produce ethanol (Vander Pol et al., 2009; Rush, 2005). This results in approximately a three-fold concentration of the non-starch components within the remaining residue (Aldai et al, 2010b; DDGS User Handbook, 2009; Gibb et al., 2008; Stock et al., 2000). Distillers by-products can then be fed at levels up to 15% of the diet dry matter (DM) to serve as a source of supplemental protein or, at levels exceeding 15%, to serve as an energy replacement for grains (Klopfenstein, 1999).

Grains are often fed to feedlot cattle because they are typically the cheapest source of energy. Because of its high digestibility, starch is assumed to be the primary source of energy and a direct indicator of the value of a cereal grain (Gibb et al., 2008). However, feeding trials with corn wet distillers grains plus solubles (WDGS) has indicated that the energy value of distillers grains is at least as high as the complete grain (Klopfenstein et al., 2008).

According to Stock et al. (2000), distillers by-products contain 25 to 32% crude protein and 13 to 16% fat (DM basis). These values are in accordance with the National Research Council (1996), who found distillers by-products to contain 30-35% crude protein, 10-15% fat, 40-45% neutral detergent fibre, and 5% ash. Wheat is similar to corn in most nutrients, but has about half the oil content and considerably more protein (National Research Council, 1996). When comparing corn to wheat DDGS, the

production of ethanol increases crude protein levels from 7.4-10% to 23-32% and fat concentration from 3.5-4.7% to 9-12% in the resulting corn DDGS, while the production of ethanol increases crude protein concentrations from 14% to 20-38% and fat levels from 1.6-2.0% to 2.5-6.7% in the resulting wheat DDGS (Klopfenstein et al., 2008; DDGS User Handbook, 2009; Aldai et al., 2010b).

The use of distillers grains also adds to the net energy value of the grain, although the energy value may be influenced by the type of grain used in the fermentation process as well as by the amount of solubles added to the distillers grain (Stock et al., 2000). In general, wet distillers by-products have up to 147% of the net energy of corn; however, drying the distillers grain reduces the net energy value (Klopfenstein et al., 2008; Stock et al., 2000; Rush, 2005). When comparing WDGS and DDGS to corn, WDGS has up to 47% greater feeding value than corn, while DDGS only has 24% greater feeding value than corn (Klopfenstein et al., 2008; Ham et al., 1994). Although the explanation for this effect is not apparent, it is possible that the heating process may result in a loss of amino acids, lowering the feeding value of DDGS when compared to WDGS; however, the particular amino acids affected are not yet known.

The use of WDGS does have associated problems that arise during handling and storage due to a reduced shelf-life of only two weeks (Bothast and Schlicher, 2005). To increase the shelf-life and reduce the transportation costs associated with hauling water, WDGS can be dried to produce DDGS. Although drying WDGS is expensive and may account for approximately 40% of the energy costs incurred by a processor, it also produces a uniform, stable, high-quality feed by-product suitable for transportation over long distances and may be essential for plant profitability (Bothast and Schlicher, 2005; Ham et al., 1994).

Several theories have been proposed to explain how distillers grains have improved feeding quality over cereal grains. Vander Pol et al. (2009) found that the elevated fat content of WDGS protects the fat present in WDGS from undergoing complete biohydrogenation. This allows for a more efficient use of the unsaturated fatty acids (UFA) by the animal because a higher concentration of UFA can reach the duodenum where it can be utilized (Vander Pol et al., 2009). Increased UFA utilization,

coupled with increased propionate production and greater fat digestibility, can explain why WDGS has a greater energy value when compared to grain (Vander Pol et al., 2009).

2.1.1 Distillers Grains as a Ruminant Energy Source

There has been a significant amount of research conducted evaluating the feeding value of corn DDGS as both a protein and energy source on finishing cattle (Ham et al., 1994; Klopfenstein et al., 2008). Larson et al. (1993) observed that when corn WDGS was fed at levels ranging from 5.2 to 40% of the dietary dry matter (DM), the net energy gain was 169 and 128% of the net energy gain of dry rolled corn, respectively. Similarly, Ham et al. (1994) found that feeding corn WDGS and DDGS at 40% of the dietary DM resulted in an increase of 39 and 21% of the net energy value of corn grain, respectively. Based on animal performance, Al-Suwaiegh et al. (2002) calculated that the net energy content of corn WDGS is 33.3% greater than that of dry-rolled corn, with 42% of the improved net energy gain being attributed to the increased lipid content in corn distillers grains.

When compared to corn distillers grains, there have been relatively few studies evaluating the feeding value of wheat distillers grains (Walter et al., 2010). McKinnon and Walker (2008) observed that replacing rolled barley with wheat DDGS at 25 and 50% of the dietary DM during backgrounding increased the average daily gain and improved feeding efficiency, while increasing the net energy gain by 8.2% relative to the control barley diet. However, Beliveau and McKinnon (2008) did not observe an effect on average daily gain, feed efficiency, or dry matter intake when wheat DDGS was substituted for rolled barley at levels up to 23% of the finishing ration. In contrast to these findings, Gibb et al. (2008) observed that feeding finishing cattle wheat DDGS up to 60% of the diet had a linear increase in dry matter intake, similar average daily gain, and poorer feed efficiency as wheat DDGS inclusion rate increased.

Walter et al. (2010) evaluated the performance of feedlot cattle fed finishing diets composed of 20 or 40% wheat or corn-based DDGS (DM basis). The results from this study indicated that feeding wheat DDGS in finishing diets up to 40% caused an increase in dry matter intake and a reduced days on feed, while supplementing corn DDGS for barley resulted in a decrease in dry matter intake and an improved gain to feed ratio

(Walter et al., 2010). These results support previous findings that showed feeding corn DDGS results in an increase in net energy (Walter et al., 2010). These findings indicated that replacing barley with corn or wheat DDGS at levels up to 40% of the ration can lead to superior animal performance.

2.1.2 Effect of Feeding Distillers Grains on Carcass Traits

At modest rates of gain, carcass fat usually increases with increased growth rates (Owens et al., 1995). This increase is typically attributed to an increase in subcutaneous fat (Benson et al., 2005). In a study conducted by Gibb et al. (2008), feeding wheat DDGS did not affect carcass weight, dressing percentage or ribeye area. However, Gibb et al. (2008) found that feeding cattle 20% wheat DDGS (DM basis) increased the amount of back fat when compared to cattle fed the barley control diet. In contrast, Gordon et al. (2002) reported that back fat levels decreased with increased levels of corn DDGS inclusion. Lodge et al. (1997) found that back fat thickness was not affected by dietary supplementation with sorghum wet or dry distillers grains.

Similar to Gibb et al. (2008), Walter et al. (2010) did not find an effect of corn or wheat DDGS inclusion on hot carcass weight, estimated lean yield, grade fat marbling scores or ribeye area. Beliveau and McKinnon (2008) also reported that including wheat DDGS at levels up to 23% of the finishing ration had no effect on estimated lean yield or marbling scores. However, Walter et al. (2010) reported a linear increase in dressing percentage with the inclusion of wheat DDGS and a quadratic increase in dressing percentage with the inclusion of corn DDGS, with a local maxima at 26% inclusion (DM). Benson et al. (2005) also observed a positive linear effect of corn DDGS on dressing percentage (up to 35%, DM), which was attributed to an increase in subcutaneous fat and poorer yield grades (Eun et al., 2009). Similarly, both Benson et al. (2005) and Eun et al. (2009) reported that yield grades increased in response to greater inclusion levels of corn DDGS, reflecting the higher levels of subcutaneous carcass fat. In contrast, Walter et al. (2010) found that including wheat and corn DDGS at levels up to 40% (DM basis) had no effect on estimated lean yield or grade fat, although the estimated lean yield decreased and grade fat numerically increased as DDGS level increased.

2.1.3 Effect of Feeding Distillers Grains on Meat Quality

Meat quality can be described as the overall characteristics of meat, including its physical, chemical, microbial, sensory, nutritional and cooking properties. Among all of the properties, colour and flavour are of major importance during a consumer's evaluation of meat quality (Luciano et al., 2009). According to Shand et al. (1998), diet can have a strong influence on beef flavour and fatty acid composition, with high energy grain diets producing a more acceptable flavour in red meats than low energy diets. In the cattle industry, a paradigm shift is occurring: in the USA producers are moving away from corn feedstock due to greater costs and reduced supplies of corn towards by-products, such as distillers grains (Depenbusch et al., 2009). In Canada, similar pressures on feed grain costs and supplies are driving the use of DDGS, with wheat being the distillers grain of choice (Aldai et al., 2010b). In terms of meat quality, there have been many studies examining the effects of feeding corn DDGS on beef meat quality; however, there is limited information regarding feeding wheat DDGS on beef meat quality.

One possible advantage of including wheat DDGS in the diet of feedlot cattle may be its ability to improve the health properties of beef fat by increasing the levels of polyunsaturated fatty acids and by improving the profile of biohydrogenation products (Aldai et al., 2010a). Unfortunately, this may also have a negative impact on the meat's susceptibility to oxidation, rancidity, colour stability, and off-flavour development (Dugan et al., 2008; de Mello Jr., 2008)

2.1.4 Effect of Lipid Oxidation on Meat Quality

Lipid oxidation is one of the main factors limiting the quality and acceptability of meats and other muscle foods (Chaijan, 2008) and has long been recognized as a leading cause of quality deterioration in muscle foods, often being the decisive factor in determining food product storage life (Ross and Smith, 2006). In red meats, biological changes that occur *post-mortem,* as well as pro-oxidative processing and storage conditions, compromise the muscle's natural antioxidant defences and favour the onset of oxidative processes (Luciano et al., 2009; Min et al., 2008). This process leads to discolouration, drip losses, off-odour and off-flavour development, texture defects, and

the production of potentially toxic compounds, such as malondialdehyde (Richards et al., 2002).

Lipid oxidation is a chain reaction consisting of initiation, propagation, and termination reactions, involving the production of free radicals (Nawar, 1996). In essence, it is a complex process whereby unsaturated fatty acids react with molecular oxygen via a free radical mechanism (Ross and Smith, 2006). Initiation occurs when hydrogen atoms at the α-methylene groups near the double bonds of unsaturated fatty acids are removed to form alkyl free radicals, which are highly reactive molecular species containing unpaired electrons that are likely to participate in chemical reactions. These radicals then undergo propagation to induce the formation of new radicals, starting the chain of lipid oxidation. Figure 2-1 diagrams a simplified three-step free-radical scheme outlining the flow of free radicals during lipid oxidation:

1. Initiation

Initiator → Free Radicals (R°, ROO°)

2. Propagation

$R° + O_2 \rightarrow ROO°$; $ROO° + RH \rightarrow ROOH + R°$

3. Termination

$R° + R° \rightarrow R\text{-}R$

$R° + ROO° \rightarrow ROOR$

$ROO° + ROO° \rightarrow ROOR + O_2$

Figure 2-1 Lipid oxidation mechanism (Nawar, 1996).

The susceptibility of meat to lipid oxidation is influenced by several factors, including the concentration of prooxidants, endogenous ferrous iron, myoglobin, pH, temperature, ionic strength, oxygen consumption reactions, and fat composition because the degree of lipid oxidation is dependent on the degree of polyunsaturation in the fatty acid profile (Luciano et al., 2009; Aldai et al., 2008; Chaijan, 2008; Min et al, 2008;

Wood et al., 2003). Muscle type also has a significant effect on a meat's susceptibility to oxidation. In general, muscles with greater proportions of red fibres, or darker muscles, are more susceptible to lipid oxidation because they contain more iron and phospholipids than muscles containing predominantly white fibres (Wood et al., 2003). These findings are supported by de Mello Jr. et al. (2007; 2008) who examined the effect of feeding beef steers corn WDGS (0, 15 or 30% DM basis) on the extent of lipid oxidation in steaks obtained from the strip loin and top blade following seven days of simulated retail display. They found that the inclusion of 30% WDGS in the diet of steers resulted in higher levels of oxidation for the top blade and strip loin steaks following seven days of retail display (de Mello Jr. et al., 2007; 2008).

Beef is susceptible to lipid oxidation because of high heme pigment content due to a greater proportion of red muscle fibres (Min et al., 2008). In addition, grinding meat results in greater lipid oxidation than leaving the meat in whole cuts because grinding incorporates oxygen into the meat, mixes reactive components, and increases surface area as a result of particle size reduction (Faustman et al., 2010).

Rates of lipid oxidation also increase with increasing temperature and time as with most chemical reactions (Kanner, 1994). Likewise, cooked meat is more susceptible to lipid oxidation than raw meat because heating disrupts the muscle cell structure, inactivating antioxidant enzymes and other antioxidant compounds (Jayathilakan et al., 2007; Min et al., 2008; Kanner, 1994). The high temperatures encountered during heating also reduce the activation energy required for the onset of lipid oxidation, decomposing the hydroperoxides to free radicals and stimulating the autoxidation process (Min et al., 2008). Furthermore, freezing can facilitate lipid oxidation due to concentration effects (Foegeding et al., 1996). Likewise, the inclusion of salt can catalyze lipid oxidation in muscle tissue by having the sodium molecule replacing iron in the cellular components via an ion exchange reaction, with the displaced iron being able to participate in the initiation of lipid oxidation (Chaijan, 2008).

2.1.4.1 Lipid Oxidation Products

Lipid oxidation generates a wide range of products that can impact meat quality and stability. Of greatest importance is the major primary product of this reaction,

hydroperoxides, which have the ability to decompose into a wide range of secondary compounds, including alkanes, alkenes, aldehydes, ketones, alcohols, esters, acids, and hydrocarbons (Ross and Smith, 2006). Of these compounds, aldehydes are considered the most important breakdown product because they are the major contributors to the development of rancid off-flavours and odours (Ross and Smith, 2006).

Lynch et al. (2001) found that propional, pentanal, hexanal, and 4-hydroxynonenal are the primary aldehydes formed during the oxidation of ground beef stored at 4°C. During lipid oxidation, these primary aldehydes are able to further react to generate a wide range of secondary aldehyde products, such as n-alkanals, trans-2-alkenals, 4-hydroxy-trans-2-alkenals, and malondialdehyde (Lynch and Faustman, 2000). These products are more stable than free radicals and have the ability to diffuse into the cellular media where they may exert toxicological effects by reacting with other biomolecules (Chaijan, 2008).

The aldehydes produced during lipid oxidation can also alter myoglobin stability because the covalent attachment of the aldehydes to oxymyoglobin (OxyMb) render the OxyMb more susceptible to oxidation (Faustman et al., 2010). Alternatively, the covalent binding of α,β-unsaturated aldehydes to OxyMb at key amino acid residues may alter the tertiary structure, increasing its susceptibility to oxidation and resulting in the brown discolouration in fresh meat (Alderton et al., 2003).

2.1.4.2 Measuring Lipid Oxidation Products

Lipid oxidation methods have been divided into those that measure changes in primary products and those that measure changes in secondary products (Gray and Monohan, 1992). Methods measuring changes in primary products quantify the loss of reactants, such as unsaturated fatty acids or oxygen, or the formation of primary oxidation products, such as hydroperoxides or conjugated dienes (Ross and Smith, 2006). Measuring the peroxide concentration is commonly used to quantify the extent of lipid oxidation; however, peroxides decompose to secondary products relatively quickly so this measure can result in an underestimation of the degree of oxidation (Ross and Smith, 2006). Therefore, methods that quantify these primary oxidation products are thought to

be more suited to measuring low levels of oxidation found in uncooked products and products stored at low temperatures (Gray and Monohan, 1992).

Quantifying thiobarbituric acid reactive substances (TBARS) is one of the oldest and most commonly used methods for assessing lipid oxidation in foods (Byrne et al., 2001). This method is based on the spectrophotometric determination of the secondary lipid oxidation product (Juntachote et al., 2006), malondialdehyde (Juntachote et al., 2007; Sorensen and Jorgensen, 1996), which is a potential carcinogen produced in greater quantities in phospholipids than in triglycerides (Hernandez et al., 1999; Min et al, 2008). These aldehydes have both cytotoxic and genotoxic properties due to their high reactivity; therefore, repeated consumption of highly oxidized meat can be a threat to human health (Min et al., 2010).

Due to the fairly simple procedure and high correlations with sensory scores, many studies have relied on TBARS for the determination of oxidative status (Ross and Smith, 2006). The major disadvantage with TBARS determination is that it is not specific for malondialdehyde so other oxidation compounds or compounds not related to lipid oxidation can react with thiobarbituric acid to yield an overestimation of the extent of lipid oxidation (Gray and Monohan, 1992). Therefore, the TBARS procedure is used to assess the total extent of lipid oxidation in general rather than being used to quantify malondialdehyde specifically (Ross and Smith, 2006).

TBARS has been used to determine the extent of lipid oxidation in beef animals fed various levels of distillers grains (De Mello Jr. et al. 2007, 2008; Gill et al., 2008; Depenbusch et al., 2009; Koger et al., 2010). Aside from Depenbusch et al., 2009, all of these researchers found that the inclusion of distillers grains into the diet of finishing cattle resulted in higher levels of oxidation following retail display. This effect was observed because meat obtained from cattle fed distillers grains contains greater levels of unsaturated fatty acids, which is one of the substrates necessary for this deteriorative reaction (Faustman et al., 2010).

2.1.4.3 Mitigating Lipid Oxidation

Antioxidants are typically added to meat to protect the meat from oxidative deterioration, improving the antioxidant status of animal tissues, thus reducing oxidation

(Haak et al., 2008). Alpha-tocopherol, also known as vitamin E, is a membrane-associated antioxidant that protects the vulnerable unsaturated fatty acids in cell membranes and plasma lipoproteins from endogenous and exogenous oxidizing agents (Guo et al., 2006). In biological systems, it is the primary lipid soluble antioxidant that acts by disrupting the chain of lipid oxidation in cell membranes, preventing the formation of lipid hydroperoxides (Haak et al., 2008). Vitamin E donates its phenolic hydrogen on the carbon six hydroxyl group to the free radical and then stabilizes the unpaired electron using its chromanol ring (Min et al., 2008). It is generally accepted that the most effective method of reducing lipid oxidation in precooked meats is accomplished by supplementing diets with vitamin E because the vitamin is incorporated into the membrane structures, thereby protecting the phospholipids against oxidative attack (Jensen et al., 1998).

Mohan et al. (1990) has shown that supplementation of animal diets with dietary vitamin E leads to elevated concentrations of α-tocopherol in the cell membranes, resulting in a lower susceptibility to oxidation. The most common form of vitamin E used for dietary supplementation is the acetate ester of all-rac-α-tocopherol (α-tocopherol acetate) because the esters are resistant to oxidation and will not undergo degradation while passing through the animal (Jensen et al., 1998). Once in the gut, the esters are hydrolysed to release the native α-tocopherol and regain its antioxidant activity (Jensen et al., 1998). In general, the greater the amount of vitamin E deposited, the better protection the muscle has against oxidative attack (Jensen et al., 1998) because α-tocopherol is not degraded during storage, thus exhibiting an ongoing protective effect (Guo et al., 2006).

2.1.4.4 Myoglobin Oxidation

Myoglobin is a globular heme protein found in the muscle of meat producing animals and is a major contributor to muscle colour, depending on its redox state and concentration (Chaijan et al., 2008). This molecule is made up of a single polypeptide chain, globin, consisting of 153 amino acids and a prosthetic heme group (Pegg and Shahidi, 1997). It is the presence of the heme group that makes myoglobin a major catalyst of lipid oxidation because as this central iron atom oxidizes, the meat undergoes discolouration, changing from red OxyMb to brownish metmyoglobin (MetMb) and, as

the ferrous (Fe^{2+}) heme iron oxidizes to its ferric (Fe^{3+}) form, oxygen is released and a superoxide anion is formed (Faustman et al., 2010). The superoxide anion rapidly dismutates into hydrogen peroxide before the oxygen molecule can react with the hydrogen peroxide and Fe^{3+} to produce a free radical and facilitate lipid oxidation (Chan et al., 1997). Furthermore, the hydrogen peroxide can react with MetMb to form the red-pigmented prooxidative ferrylmyoglobin radical (Min et al, 2008).

Ferrylmyoglobin is a relatively stable species that is slowly reduced back to MetMb at physiological pH; however, it is responsible for the oxidation of a variety of substrates (Chaijan et al., 2008). Under the conditions found in fresh meat (pH 5.5 to 5.8), ferrylmyoglobin is able to initiate lipid oxidation by replacing the hydrogen atoms present on a fatty acid chain with a stereo specific oxygen molecule (Chaijan et al., 2008). Ferrylmyoglobin formation in muscle tissue is also determined by the production of hydrogen peroxide and its potential to oxidize lipids is dependent on the reducing agent concentration and their compartmentalization in the muscle cells (Baron and Anderson, 2002).

2.1.5 Effect of Meat Colour on Meat Quality

Food quality is the sum of three principle components: nutritional value, safety, and consumer acceptability (Clydesdale, 1978). Consumer acceptability includes a large array of sensory attributes, including visual appeal, aroma, flavour, texture and mouth feel (Bekhit and Faustman, 2005). Clydesdale (1978) argued that colour is perhaps the most important sensory attribute of fresh foods because if it is deemed unacceptable then the food will not be purchased and/or eaten and, consequently, all other sensory attributes lose significance.

The colour that consumers associate with desirable meat quality is species dependent. For example, chicken, turkey and pork meat require a greyish-pink colour to be considered normal while fresh lamb and beef products require a bright red colour to be desirable (Bekhit and Faustman, 2005). If the colour of fresh beef is not a bright cherry-red then the meat may be considered undesirable or even spoiled (Hood and Riordan, 1973). The economic importance of meat colour deterioration was highlighted by Williams et al. (1992), who estimated that an average loss of sales due to colour

deterioration was 3.7% for an entire meat department and 5.4% for fresh meats. It was concluded that increasing the case life of meat by 1-2 days could save the US industry up to one billion dollars annually (Williams et al., 1992).

The biochemical basis of the red colour in meats is well established and is dependent on the concentration and redox state of myoglobin, haemoglobin and cytochromes in meat (Bekhit and Faustman, 2005). However, meat colour is also influenced by the beef surface characteristics related to final pH, the structure of the muscle surface, and marbling (Beriain et al., 2009; Luciano et al., 2009; Mohomed et al., 2008; Brewer, 2004; Insausti et al., 1999).

As previously mentioned, myoglobin is an intracellular globular haem protein found in skeletal and cardiac muscle that is responsible for reversibly binding oxygen, for providing oxygen storage, and for facilitating the diffusion of oxygen from the extracellular space to the mitochondria (Bekhit and Faustman, 2005). In meat, myoglobin is found in three forms: oxymyoglobin, deoxymyoglobin and metmyoglobin, with the relative proportions of these myoglobin forms determining the colour of fresh meat (Bekhit and Faustman, 2005). In its native physiological state, myoglobin has the ability to become oxygenated to form oxymyoglobin, thus forming a bright red colour. This state of myoglobin absorbs less light than reduced myoglobin, thus, producing redder meat with a higher *a** value (Gatellier et al., 2005). Oxymyoglobin is also capable of undergoing reverse oxygenation. Under low oxygen conditions, myoglobin exists with iron in the reduced (Fe^{2+}) state so oxymyoglobin releases the oxygen molecule and reverts to the deoxygenated form, producing meat that is visually dark red-purple (Brewer, 2004). Once the iron atom present in myoglobin or oxymyoglobin is converted from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state, the molecule can no longer bind oxygen, so it is converted to metmyoglobin. This results in meat that is visually brown or grey-brown and is often associated with higher b* values (Brewer, 2004).

Since the proportion of the different states of myoglobin at the meat surface changes with storage and the surrounding atmosphere, this has a large impact on the meat's shelf life (Beriain et al., 2009). According to Beriain et al. (2009), meat discolouration is defined as "divergence from the consumer-defined ideal to something less desirable, such as cherry red to brown." Meat discolouration, due to the formation of

metmyoglobin, is often considered an indicator of inferior or old meat (Kim et al., 2009). For example, Hood and Riordan (1973) found a 50% reduction in retail sales of beef when metmyoglobin formation reached 20% of the total pigment present.

Colour evaluation is especially important while evaluating the effects of feeding distillers grains on beef meat quality. For example, during instrumental colour evaluation of strip loin steaks obtained from steers fed various levels of corn wet (WDG) or corn dry (DDG) distillers grains, Roeber et al. (2005) noticed steaks from steers fed 20% DDG, 40% DDG, and 40% WDG were lighter (higher L* value) than steaks from steers fed 10% of either the DGS or WDG diets following retail display. They also noticed steaks from steers fed the control diet and steaks from steers fed 10% DDG and 10% WDG were redder (higher a* value) than steaks from steers fed higher concentrations of distillers grains (Roeber et al., 2005). With these findings, Roeber et al. (2005) were able to conclude that diet supplementation with 40-50% DM WDG or DDG results in declining colour stability of strip loin steaks; distillers grains supplementation at lower levels (10-25% DM) maintains or slightly enhances the shelf life of strip loin steaks with no effect on palatability. These findings can have huge implications for the retail beef sector because an observed reduction in the shelf-life of fresh meat could cost both the retail chain and consumer money due to discolouration.

2.1.6 Effect of Tenderness on Meat Quality

Tenderness is a highly valued consumer trait and, from a physical standpoint, is dependent on the architecture and the integrity of the skeletal muscle cell, the activity of endogenous proteases such as calpain within the muscle cell, and the extracellular matrix (McCormick, 2009). Muscle cells are among the most highly organized cells in the animal body because they perform a vast array of mechanical functions, ranging from the movement of limbs for locomotion or other gross movements to finer tasks, such as maintaining balance and coordination (Huff-Lonergan et al., 2010).

On the molecular level, muscle cells have a striated appearance due to specialized cylindrical organelles called myofibrils (Huff-Lonergan et al., 2010). Myofibril proteins are further made up of repeating units called sarcomeres, which contain all of the structural elements necessary to perform the physical act of contraction and are

responsible for the striated appearance of the muscle cell (Fraterman et al., 2007). These striations arise from alternating protein dense A-bands and less dense I-bands (Huff-Lonergan et al., 2010). The I-bands are further bisected with dark lines known as Z-lines, with the distance between two Z-lines being the sarcomere (Huff-Lonergan et al., 2010).

When evaluating the appearance of the bands, it is apparent that the I-band is comprised of thin filaments while the A-band is comprised of thick filaments and some overlapping thin filaments (Goll et al., 1984). The backbone of the thin filament is further comprised of the protein actin, while the majority of the thick filament is comprised of the protein myosin. Myosin contains a tail region that forms the backbone of the thick filament, and a globular head region that protrudes from the thick filament and interacts with actin in the thin filament (Huff-Lonergan et al., 2010). During contraction in living muscles, ATPase activity of myosin provides energy for myosin bound to actin to swivel and pull the thin filament towards the centre of the sarcomere, shortening the myofibril, the muscle cell, and eventually the muscle cell to produce contraction (Goll et al., 1984). However, in post-mortem muscle, calcium leaches from the sarcoplasmic reticulum into intercellular space, activating actomyosin ATPase (Hwang et al., 2003). Once the supply of ATP is depleted, the resulting actin-myosin (actomyosin) bonds become permanent due to the development of rigor bonds (Huff-Lonergan et al., 2010). In meat, the amount of myofibril shortening will have an influence on the overall tenderness because meat with very short sarcomeres tends to be tougher (Locker & Hagyard, 1963).

During post-mortem aging, the presence of calcium also activates calpains, the calcium-dependent peptidases (Ouali et al., 2006) which can lead to increased tenderness. The calpain system is composed of several isoforms of the proteolytic enzyme calpain, with the two best-characterized isoforms being μ-calpain and m-calpain (Huff-Lonergan et al., 2010). These two proteins are involved in the proteolysis of the cytoskeletal proteins, titin and nebulin, and the intermediate filaments, desmin, to initiate myofibrillar protein degradation (Huff-Lonergan et al., 2010). This effect is influenced by the rate of pH decline, which plays an important role in the rate of proteolysis of myofibrillar proteins by calpain (Huff-Lonergan et al., 2010). The accelerated decline of early post-mortem pH appears to favour accelerated autolysis and activation of μ-calpain as well as accelerated proteolysis of known caplain substrates, accelerating the tenderization

process and, in some cases, positively influencing water holding capacity (Melody et al., 2004).

In order to evaluate the tenderness of meat, two techniques are commonly employed: instrumental analysis to delineate the physical properties of the meat, and sensory evaluation using human subjects to evaluate sensory and physical properties (Ross, 2009). Traditionally, these two techniques were used separately; however, when used in combination, sensory evaluation and instrumental analysis provide a great deal of information and a more complete picture of the product (Ross, 2009).

2.1.6.1 Objective Tenderness Evaluation

Warner-Bratzler (WB) shear force provides an objective assessment of meat tenderness (Shackelford et al., 1991); however, the protocols for measuring WB shear force vary widely amongst institutions, with this variation affecting the repeatability of shear force evaluations among institutions (Wheeler et al., 1996). Although shear force methods vary among institutions, WB shear force values have a strong relationship with trained sensory panel tenderness ratings when measurement protocols are properly executed (Shackelford et al., 1997).

Reports of the consumer threshold for acceptability in tenderness measured by WB shear force vary among researchers (Gill et al., 2008). Shackelford et al. (1991) identified the American consumer threshold for "slightly tender" in retail food services to range from 3.9 to 4.6 kg shear force. However, some consumers have identified beef samples with WB shear force values of 2.3 kg to be unacceptable, while others have identified beef samples with WB shear force values of 4.5 kg to be acceptable (Savell et al., 1987).

Several studies have been conducted to evaluate tenderness differences in meat cuts obtained from beef animals fed different distillers grains diets. Gill et al. (2008) reported no observable differences in meat obtained from crossbred beef steers fed diets containing corn or sorghum distillers grains following 11 days aging. Roeber et al. (2005) obtained similar results during tenderness evaluation following the addition of varying levels of WDG or DDG to the diets of finishing Holstein steers following 13 days aging. These results are also in agreement with those of Koger et al. (2004), who did not find a

difference in WBSF values amongst cattle fed a control finishing diet or diets containing 20 and 40% WDG or DDG following 14 days aging.

Likewise, Shand et al. (1998) and Aldai et al. (2010b) did not detect significant differences in WB shear force values during the evaluation of meat obtained from beef animals fed wheat-based distillers grains (aged 7 and 20 days, respectively). Aldai et al. (2010b) did report that meat from animals fed a barley-based control diet to be less tender (highest proportion of tough shears at day two and lowest proportion of tender shears at twenty days) than meat from animals fed a distillers grain diet.

Recent literature also supports the findings of previously published work. Leupp et al. (2009) did not find any differences in WBSF values between crossbred steers fed a control diet or 30% corn DDGS during either the growing period, the finishing period, or both the growing and finishing periods. Similarly, Koger et al. (2010) failed to find any differences in WBSF values for Angus crossbred steers fed 20% or 40% corn WDGS or corn DDGS. These studies indicate that feeding beef cattle a DDGS diet will not impact WB shear force values; however, little work has been conducted on the effect of feeding wheat DDGS on WB shear force values.

2.1.6.2 Subjective Tenderness Evaluation

Sensory evaluation, as defined by the Sensory Evaluation Division of the Institute of Food Technologists, is the scientific discipline used to "evoke, measure, analyze and interpret reactions to those characteristics of food as they are perceived by the senses of sight, smell, taste, touch and hearing" (Ross, 2009). This relays vital information to the researcher regarding consumer product acceptability, quality control, storage and shelf-life, processing changes, product development and product reformulation (Ross, 2009).

Sensory evaluation of meat products can provide researchers with important information regarding tenderness, juiciness, flavour intensity, amounts of connective tissue, flavour desirability and overall palatability as well as information regarding any flavour or texture descriptors. Early research conducted by Shand et al. (1998) focussed on the eating quality of beef from steers supplemented with wet brewers grains (WBG) or wheat-based WDG during the backgrounding and finishing phases of a feedlot trial. They noted no significant differences in any of the sensory properties of loin roasts due to diet

and concluded that the eating quality of beef loin roasts from steers fed either conventional barley-based, WBG, or wheat WDG-based rations during backgrounding and finishing trials were very similar (Shand et al., 1998).

Other work conducted by Roeber et al. (2005) utilized a ninety-five person consumer panel to evaluate the effect of feeding up to 50% corn-based DDG or WDG on the sensory traits of beef obtained from Holstein steers. Evaluation of strip loins showed a trend for steaks obtained from steers fed 25% WDG receiving the highest, and steaks from steers fed 50% WDG receiving the lowest numerical tenderness and juiciness like/dislike scores, although flavour like/dislike ratings did not differ among dietary treatments (Roeber et al., 2005). Roeber et al. (2005) did notice a surprisingly high percentage of consumers who were dissatisfied with tenderness, which was attributed to the halo effect, which is the consumers' inability to separate tenderness, juiciness and flavour completely.

Gill et al. (2008) conducted a consumer panel in a similar manner to Roeber et al. (2005), in which ninety-six panellists evaluated strip loins obtained from cross-bred yearling steers fed steam-flaked corn diets supplemented with 15% corn or sorghum DDGS or WDGS. Like Roeber et al. (2005), no differences were found between treatments with respect to flavour and juiciness; however, steaks obtained from steers fed corn distillers grains were more tender than steaks obtained from steers fed sorghum distillers grains (Gill et al., 2008).

Other researchers have utilized trained sensory panels to evaluate differences in meat quality from beef cattle fed various distillers grains diets. Depenbusch et al. (2009) had their sensory panel evaluate ribeye steaks from yearling heifers fed finishing diets based on steam-flaked corn containing up to 75% corn-based DDGS. Results from the trained sensory panel indicated a linear increase in myofibrillar and overall tenderness as dietary inclusion of DDGS increased from 0 to 75%, which they attributed to a linear decrease in connective tissue amount as a result of lighter and less finished carcasses for heifers fed high levels of DDGS (Depenbusch et al., 2009). They also observed that beef flavour intensity was greatest among heifers fed 45 and 60% DDGS and lowest for heifers fed 0% DDGS, despite the fact that differences in marbling were not observed (Depenbusch et al., 2009). The findings by Depenbusch et al. (2009) contrast those of

Roeber et al. (2005) and Gill et al. (2008), who reported similarities in meat flavour and tenderness for cattle finished on diets with or without distillers grains.

The Leupp et al. (2009) trained sensory panel evaluating the *longissimus* muscle from crossbred and purebred steers produced results that both agreed and contrasted with the results reported by Depenbusch et al. (2009). Unlike Depenbusch et al. (2009), Leupp et al. (2009) sensory panel indicated no tenderness differences, but they did find steaks from steers fed 30% DDGS during the finishing period to be juicier than control steaks. However, both Depenbusch et al. (2009) and Leupp et al. (2009) noted that steers fed DDGS were more flavourful than control steaks.

The only study evaluating the effects of feeding wheat DDGS on beef meat quality was conducted by Aldai et al. (2010b). In this study, crossbred beef steers were fed corn or wheat DDGS at levels of 20 or 40% of the dietary dry matter intake and the resulting *longissimus* muscle was evaluated by trained sensory panellists (Aldai et al., 2010b). In general, they found steaks from steers fed DDGS were rated highest for flavour desirability than steaks from steers fed the control diet (Aldai et al., 2010b). More specifically, the flavour attributes of steaks from steers fed 20% and 40% corn DDGS differed significantly, with steaks from steers fed 20% corn DDGS being rated higher for beef flavour intensity, flavour desirability, and overall palatability (Aldai et al., 2010b). Steaks from steers fed corn DDGS also had higher initial and overall tenderness scores and were perceived to have lower amounts of connective tissue than steaks obtained from steers fed the control barley-based diet (Aldai et al., 2010b).

In the study conducted by Aldai et al. (2010b), the relationship between the inclusion of dietary corn DDGS in the steers' diet and improved tenderness in the resulting meat was not clear but it was hypothesized that the relationship links to thicker backfat present in animals fed corn DDGS and, thus, to a slower rate of cooling that may have accelerated or maintained post-mortem muscle metabolic activity, leading to increased tenderization (Aldai et al., 2010b).

2.1.7 Effect of Fatty Acid Composition on Meat Quality

Current interest in meat fatty acid composition stems from the need to find ways to produce healthier, more stable meat (Wood et al., 2003). Healthier meat is obtained

with a higher ratio of PUFA to saturated fatty acids (≤0.4) and a more favourable balance between n-6 and n-3 PUFA (<4.0) (Warren et al., 2008). This is often difficult to achieve because adverse effects on meat quality are observed when concentrations of α-linolenic acid (C18:3) approach 3% of the lipid fraction (Warren et al., 2008).

Linoleic acid is the primary n-6 fatty acid and α-linolenic acid is the primary n-3 fatty acid found in the dietary ingredients fed to cattle (Gill et al., 2008). Linoleic acid is needed for the synthesis of proinflammatory eicosanoids (Akoh and Min, 2002), while increasing the linoleic acid content in the human diet has been found to lower blood cholesterol and to reduce the risk of coronary heart disease (Zock and Katan, 1998). In contrast, α-linolenic acid is of importance because it moderates inflammation by competing with the n-6 fatty acids (MacRae et al., 2005).

Ruminant meat, in particular, is a good source of n-3 PUFA due to the presence of α-linolenic acid in grass. Studies have shown that pasture feeding increases the levels of polyunsaturated fatty acids in the muscles of cattle above the levels encountered in grain-fed cattle (Yang et al., 2002) due to forages containing higher concentrations of α-linolenic acid (Gill et al., 2008). However, meats containing greater concentrations of unsaturated fatty acids are more prone to lipid oxidation than those that are more saturated (Yang et al., 2002), thus, more susceptible to quality deterioration.

The presence of the rumen makes fatty acid manipulation more difficult in ruminants than fatty acid manipulation in monogastric animals (Wood et al., 2003). One way manipulation can be achieved is by feeding a high-concentrate diet, which results in decreased ruminal biohydrogenation and, thus, greater concentrations of unsaturated fatty acids in the muscle tissue (Vander Pol et al., 2009). Larick and Turner (1989) observed differences in the linoleic, palmitic and α-linolenic content in the phospholipid and polar lipid fractions of beef cattle fed various levels of wheat- and corn-based finishing diets, which they attributed to the composition of the lipid component of the grain fed to the animals. This supports the findings of Blank (1955), who discovered the lipid fraction of ground corn to contain a higher percentage of oleic and linoleic acid and a lower percentage of palmitic and α-linolenic acid than wheat flour.

Shand et al. (1999) noted steers fed wheat WDG had higher levels of saturated fatty acids relative to barley fed steers, although wheat WDG contains higher amounts of

unsaturated lipids. In contrast, Aldai et al. (2010a) found backfat levels of total saturated fatty acids from beef steers fed a control barley-based finishing diet to be unaffected by substituting barley with corn or wheat DDGS. They also found the monounsaturated fatty acid content was higher in the control samples, while the PUFA content was higher in the DDGS samples (Aldai et al., 2010a). The increased levels of PUFA encountered in the DDGS samples is congruent with previous work conducted by Schingoethe et al. (1999), who determined an inclusion of 31.2% corn WDG to a corn silage-based diet provided an additional 2% long chain and unsaturated fatty acids to the muscle tissue, mainly due to an increase in oleic and linoleic acids.

Gill et al. (2008) obtained similar results to Aldai et al. (2010a) while analysing the impact of beef cattle diets containing corn or sorghum distillers grains on the fatty acid profile of the resulting meat. They observed steers fed distillers grains had a greater concentration of n-6 fatty acids than steers fed the steam-flaked corn control diet, mainly due to an increase in linoleic acid (Gill et al., 2008). De Mello Jr. et al. (2008) also observed feeding increasing levels of WDGS significantly increased the PUFA content. As in previous studies, the increased PUFA levels were attributed to increased levels of linoleic acid in the lipid fraction (de Mello Jr. et al., 2008).

2.1.7.1 *Trans* Fatty Acids and Conjugated Linoleic Acid

When rumen bacteria isomerize and hydrogenate dietary PUFA, metabolic intermediates can accumulate (Aldai et al., 2010a). These include rumenic acid (9*c*,11*t*-18:2) and its precursor vaccenic acid (11*t*-18:1), the common intermediate produced from the biohydrogenation of linoleic acid (18:2) (Gill et al., 2008), which have been shown to demonstrate known health benefits in animal models when consumed (Collomb et al., 2006; Belury, 2002). In contrast, 10*t*-18:1 has been shown to have a negative impact on plasma cholesterol concentrations in animal models (Bauchart et al., 2007) and with coronary heart disease in humans (Hodgson et al., 1996). The *trans*-18:1 isomer profile in beef can be influenced by diet, with diets high in rapidly fermenting carbohydrates producing high levels of 10*t*-18:1 (Dugan et al., 2007; Hristov et al., 2005), while cattle on forage have been shown to increase vaccenic acid production relative to 10*t*-18:1 (Aldai et al., 2008). Therefore, finishing cattle on DDGS in place of ground barley would

lower dietary starch levels, subsequently increasing PUFA levels available for biohydrogenation, while increasing the crude fibre levels, which may elevate rumen pH and favour the production of 11*t*-18:1.

Increases in the total *trans*-MUFA have been observed in duodenal digesta (Vander Pol et al., 2009) and in strip loin steaks when feeding DDGS derived from corn, with these differences possibly being related to their greater oil content (increased from 1.9 to 3.7%; Dugan et al., 2010). Aldai et al. (2010a) observed that feeding increasing levels of corn DDGS resulted in a linear increase in total *trans*-fatty acids. Likewise, Dugan et al. (2010) observed that feeding increasing amounts of wheat DDGS led to a linear reduction in 10*t*-18:1 and linear increases in several other *trans*-18:1 isomers, which included 11*t*-18:1, in the brisket fat composition of British crossbred heifers.

However, the relative flow of PUFA through the major biohydrogenation pathways can be evaluated by the 11*t*-/10*t*-18:1 ratio, with a higher ratio denoting improvements in its healthfulness to consumers (Aldai et al., 2010a). Aldai et al. (2010a) found that feeding steers wheat DDGS resulted in the most desirable ratio in the back fat; however, the ratio (0.58) contrasts to values of over 6 that occur in cattle fed diets containing high levels of forage (Aldai et al., 2008).

Similar to PUFA concentrations, Dugan et al. (2010) found that feeding increasing amounts of wheat DDGS led to a trend for a linear increase in total conjugated linoleic acid (CLA), which was mainly attributed to a linear increase in 9*c*,11*t*-18:2, in the brisket fat of feedlot heifers. Similar results were obtained by Depenbusch et al. (2009), who found that concentrations of CLA linearly increased in the intramuscular fat of cooked *longissimus* steaks as dietary levels of corn DDGS increased from 0 to 75%. CLA is a product of ruminal biohydrogenation of polyunsaturated fatty acids (Kelly et al., 1998) and has been shown to protect against cancer, inflammation and diabetes in experimental animal models (Belury, 2002; Pariza et al., 2001), while some recent studies have suggested that it reduces the risk of breast and colorectal cancers (Larsson et al. 2005; Aro et al. 2000) and acute myocardial infarctions in humans (Warensjo et al., 2004).

As previously mentioned, rumenic acid (9*c*,11*t*-18:2), which is derived from vaccenic acid (11*t*-18:1), has been demonstrated to have health benefits (Collomb et al.,

2006). Not all CLA isomers have a positive impact on human health. Early research conducted by McGuire and McGuire (2000) showed that 10*t*,12*c*-18:2 has the potential for beneficial human health because it has been shown to hinder obesity by inhibiting lipogenesis. However, recent work by Kennedy et al. (2011) has shown 10*t*,12*c*-18:2 to increase inflammation and insulin resistance in human adipocytes by increasing calcium levels.

2.2 Value-Added Meat Products

2.2.1 Roast Beef

Approximately 26% of the beef carcass is utilized as steaks from the loin and rib, while a larger proportion of the remaining cuts from the carcass is utilized for the production of ground meat, a lower value product (Aberle et al., 2001; Boles and Swan, 2002b). On a larger scale, only 25% of the beef produced is further processed, whereas 70% of the pork and chicken produced is further processed (Industry, Science and Technology Canada, 1993). Among these further processed goods, beef roasts are a popular food service item (Boles and Swan, 2002a). Generally, inside rounds (*semimembranosus* and *adductor* muscles) with or without cap (*gracilus* muscle) are utilized for the production of beef roasts because they have less marbling, which can interfere with cooked product appearance (Boles and Swan, 2002a).

2.2.2 Brine Injection

The beef industry has adopted moisture enhancement of fresh meat to help ensure product consistency, juiciness, and tenderness (Pietrasik and Janz, 2009). This is routinely practised by injecting various salt and phosphate formulations into the primal meat cuts (Dhanda et al., 2002). The most commonly used ingredients for this action are sodium chloride and alkaline phosphates, which synergistically act to increase water retention of meat products and to assist with the binding of water naturally present in the meat as well as any added water present in marinade solutions, thus ensuring a juicier cooked product (Pietrasik and Janz, 2009). An important characteristic of alkaline phosphates is their ability to move the meat pH away from the isoelectric range of the meat protein, increasing the proportion of negative charges on the meat proteins, thus

improving the meat's water holding and binding capacity (Pietrasik and Janz, 2009). Salts, on the other hand, increase the ionic strength of the injected solution, thereby increasing the number of hydrophilic protein interactions within the meat, which leads to increased binding of free water (Pietrasik and Janz, 2009).

When brine is injected into a meat product, the pH of the injected muscle section becomes significantly higher than the pH of the non-injected muscle section (Dhanda et al., 2002). Several authors have reported a significantly higher pH in beef roasts injected with salt/phosphate brine than the non-injected roasts (Farouk and Swan, 1997; Pietrasik and Shand, 2003; Pietrasik et al., 2005; Vaudagna et al., 2008; Pietrasik and Janz, 2009). This is beneficial for moisture enhancement systems because the higher the pH, the further away from the isoelectric point of muscle proteins and the greater the retention of water due to an increased net protein charge (Pietrasik and Janz, 2009). Within muscles, water-binding capacity is lowest at 5.4, the isoelectric point of actomyosin, but, as the muscle pH is increased, thaw loss decreases until a minimum purge is reached at pH 6.4 (Deatherage and Hamm, 1960). Oreskovich et al. (1992) noticed this phenomenon when they reported an increase in the percentage of bound water as the meat pH moved away from the isoelectric point of the predominant meat proteins.

Brine injection also affects the cooking yield and purge loss of injected meat cuts. Boles and Shand (2001) reported a significant increase in cooking yield in roast beef with brine injection. However, brine concentration and brine formulation also impact cook and purge losses of injected samples. Pietrasik and Shand (2003) noted higher cook losses for beef rolls injected with a 50% brine solution than those injected with a 25% brine solution. This occurred because the meat protein matrix could not hold most of the added water due to limited functionality of the myosin heat-set matrix caused by an unfavourable moisture/protein ratio (Pietrasik and Shand, 2003).

Likewise, Pietrasik and Janz (2009) found that increasing the salt concentration in the injected muscle from 0.5% to 1.5% yielded lower purge and cooking losses and a higher water holding capacity. This occurred because increasing the salt concentration in the water phase increased protein extraction. An increase in the extracted protein content caused an increase in the number of locations in the polypeptide chain capable of

interacting during heating and, as a result, a much more stable meat protein matrix was formed, leading to a smaller release of water and fat (Trout and Schmidt, 1984; 1986).

2.2.3 Freezing Whole Muscle Foods

Freezing is a preservation method used to store meat for relatively long periods of time. The utilization of frozen products offers the advantages of increased storage time, a greater flexibility in inventory, and greater product control (Pietrasik and Janz, 2009). Freezing and frozen storage can also affect the structural and chemical properties of muscle foods, often resulting in changes in muscle fibres and changes in the lipid and protein fractions (Pietrasik and Janz, 2009). During the freezing process, free water is expelled by osmosis into the extracellular space, forming ice crystals that later cause juice loss from the meat during thawing (Polymenidis, 1978).

Although freezing enhances shelf-life and offers greater product control, it also lowers total extractable proteins from the meat, thus, reducing meat functionality (Awad et al., 1968). Nonetheless, many roast beef processors utilize frozen imported cuts, such as the *semimembranosus*, to obtain a consistent meat supply to minimize cost fluctuations, and to help balance the supply from the local market (Boles and Swan, 2002a).

2.2.4 Thawing Whole Muscle Foods

HACCP plans require thawing and cooking regimes for the manufacture of roast beef to be approved; however, processors have many options available in order to meet specifications (Boles and Swan, 2002a). For example, processors may thaw products using air or water, although air thawing is very slow and can result in more purge than water thawing (Boles and Swan, 2002b). Boles and Swan (2002a) examined the effect of thawing injected *semimembranosus* muscles in both air and water systems. They found *semimembranosus* muscles thawed in water had significantly lower purge losses than those thawed in air; however, air-thawed muscles absorbed and retained more brine and had higher cook yields than water-thawed muscles (Boles and Swan, 2002a). The lower purge losses during thawing were attributed to a faster thawing rate in water, which minimized tissue damage and allowed the meat to retain its water holding capacity (Boles

and Swan, 2002a). In contrast, the slower air-thawing may have damaged the ultra structure of the meat, allowing for a better interaction between the muscle proteins and the salt and phosphate components in the brine (Boles and Swan, 2002a). Because the air thawed meat lost more moisture via purge, there were more charged areas available to hold the additional liquid (Hamm, 1986), hence the increased cook yield and brine retention. However, differences in protein loss during thawing were not measured in the study so it is not clear if protein loss had a significant impact on brine retention.

2.2.5 Cooking Whole Muscle Foods

Cooking is energy intensive and has a major influence on meat tenderness because water- and fat- binding properties, as well as texture, are closely related to heating conditions (Pietrasik et al., 2005). Textural changes encountered during heating are due to myofibrillar and connective tissue proteins (mainly collagen) (Bouton and Harris, 1972). When myofibrillar proteins are exposed to thermal energy they undergo denaturation, which generally leads to coagulation and loss of protein solubility (Pietrasik et al., 2005). When collagen and the surrounding connective tissue are heated, the collagen swells in the presence of moisture and then shrinks so, with extended heating in the presence of moisture, collagen becomes more tender due to further hydration and hydrolyzation (Ledward, 1984).

All of the changes that occur during cooking are temperature dependent. For example, changes in whole meat toughness at temperatures below 60°C are the result of alterations in the mechanical properties of the perimysium connective tissue, while increased toughness between 65 and 80°C are a result of changes in the myofibrillar structure (Pietrasik et al., 2005). When meat is heated between 60 and 90°C, a loss of water holding capacity occurs (Hamm, 1986), while heating to 70°C results in collagen shrinkage (Judge et al., 1984), so cooking above 70°C promotes collagen shrinkage, reduced water holding capacity, and increased cook loss (Boles and Swan, 2002a).

Several studies have been conducted to evaluate the effect of cooking regime on product quality. Ray et al. (1983) found low temperature cooking (47°C for the first hour then increased 5.6°C/hour through the fifth hour to 69°C) in water produced more tender beef roasts than high temperature cooking (58°C for the first hour then increased

5.6°C/hour through the third hour to 69°C) while retaining similar cook yields. Other work reported that cook rate did not affect tenderness but did affect cook yield, as cook yield increased with cook rate (Shin et al., 1992). In contrast, Powell et al. (2000) showed that a slower cooking rate increased the tenderness of roast beef but had no effect on overall cook yield.

Dhanda et al. (2002) evaluated the effects of cooking with moist or dry heat to two different endpoint temperatures (71 and 77°C) on the cook yield and tenderness of bison *semimembranosus* roasts. They found cooking by moist heat to an internal temperature of 71°C significantly lowered cooking losses and resulted in more tender meat due to increased moisture retention than cooking with dry-heat or cooking to an endpoint temperature of 77°C (Dhanda et al., 2002).

Similarily, Boles and Swan (2002a) cooked roasts prepared from inside rounds to an internal temperature of 63 or 80°C in either constant-temperature water baths or water baths where a 10°C difference was maintained between the roast and the water. This experiment revealed that roasts cooked more rapidly (constant-temperature) had higher cook yields than roasts cooked by the slower change in temperature method (Boles and Swan, 2002a).

Lastly, Pietrasik et al. (2005) examined the combined effects of marination and cooking regime on the cook yield and palatability of bison and beef top round roasts. The roasts were injected with a marinade to achieve 20% extension by weight and then subdivided for cooking using four cooking regimes: cooking at a constant temperature of 75°C (control); holding the roasts at an internal temperature of 55°C for 45 minutes or 90 minutes before cooking to a final temperature of 75°C; or initial cooking at 55°C, with a 5°C increase in oven temperature every hour until an internal temperature of 71°C was reached (Pietrasik et al., 2005). They found marination by injection improved the yield and tenderness of beef and bison *semimembranosus* roasts, while holding the roasts at 55°C for 90 minutes improved tenderness (Pietrasik et al., 2005).

3.0 A COMPARISON OF WHEAT- , CORN- AND WHEAT/CORN-BASED DISTILLERS GRAINS PLUS SOLUBLES ON THE QUALITY OF BEEF *LONGISSIMUS*

3.1 Abstract

This study investigated the effect of feeding crossbred beef steers diets with barley (control) or 40% dried distillers grains plus solubles (DDGS) diets derived from wheat, corn or their combination on meat quality attributes of the *longissimus* muscle. Six-bone rib sections (7th to 12th ribs) from 80 steers (20 per diet) were aged for 14d. No differences ($P>0.05$) were observed in meat composition, pH, drip loss or shear values due to dietary regime. Diet did have an effect on colour attributes, with steaks from steers fed wheat DDGS being lighter (higher L* value; $P<0.05$) than steaks from steers fed the other treatments. Similarly, retail display panel results showed wheat DDGS steaks having a lighter ($P<0.001$) lean colour score than steaks from the other diets. It was also observed that steaks from animals fed any DDGS diet lost redness faster over time (lower a* values) and had a less desirable retail appearance than control steaks ($P<0.001$). Differences were also observed in both the subcutaneous and intramuscular fatty acid profiles, especially with respect to increased *trans*-monounsaturated fatty acid levels in steers fed corn DDGS and increased levels of branched-chain fatty acids and conjugated linoleic acid in steers fed a DDGS diet. Although the fatty acid composition from cattle fed a DDGS diet had higher concentrations of PUFA, no differences were observed in regards to the oxidative stability of raw or cooked meat. Overall, feeding 40% wheat DDGS, 40% corn DDGS or their blend did not significantly affect meat quality; however, wheat DDGS did offer enhanced colour stability and a more desirable fatty acid profile over corn DDGS.

3.2 Introduction

Western Canada is experiencing a significant increase in wheat-based ethanol production. The production of biofuels as a renewable energy source has been undertaken

in an effort to lower dependence on fossil fuels (Aldai et al., 2010b). As a result, North America is experiencing the generation of large volumes of dried distillers grains plus solubles (DDGS) that can be used as a feedstock for livestock. Depending on market conditions, wheat and/or corn DDGS may be available. Considerable research has been conducted on the impact of feeding corn DDGS on beef quality; however, little is known about the effects of feeding wheat DDGS on beef quality.

The production of ethanol utilizes the starch fraction of the seed. Starch accounts for approximately 70% of the weight of wheat and, in ethanol production, this starch is removed through fermentation by yeast to produce ethanol, resulting in approximately a threefold increase of the non-starch components in the DDGS (Gibb et al., 2008). Wheat is similar to corn in most nutrients, but has about half the oil content and considerable more protein (National Research Council, 1996). During the production of wheat DDGS, protein levels were found to increase from 14% to 20-38% and fat levels were found to increase from 1.6-2% to 2.5-6.7%, depending on the feedstock (Mustafa et al., 2000). Early work conducted by Shand et al. (1998) found carcass traits, raw proximate composition and fatty acid composition of the *longissimus* muscle to be unaffected by dietary supplementation of wheat-based DDGS during backgrounding and finishing of beef steers. Differences in meat colour were observed by Roeber et al. (2005), who found that replacing 40-50% of dietary DM with corn wet or dry distillers grains resulted in the declining colour stability of strip loin steaks from finished Holstein steers; however, when corn dry distillers grains was included at 10-25% dietary DM, the shelf life of steaks was maintained or slightly enhanced, with no effect on palatability. In contrast, Aldai et al. (2010b) found that meat obtained from steers finished on corn DDGS (20 or 40% DM basis) was lighter in colour and more tender than meat obtained from steers finished on a standard barley-based finishing diet, with meat from steers fed wheat DDGS displaying intermediate characteristics between steers fed the control and corn DDGS diets. In general, very little research regarding the impact of wheat DDGS on carcass and meat quality has been published (Beliveau and McKinnon, 2008).

The decision to include DDGS in cattle diets will be based on its cost relative to feeding value and the impact that it will have on overall carcass value and meat quality when compared to other feedstuffs (Aldai et al., 2010b). One advantage of including

wheat DDGS in the diet of feedlot cattle is its ability to improve the health properties of beef fat by creating a fatty acid profile more favourable to human health than the fatty acid profile of cattle fed corn DDGS (Aldai et al., 2010a). This is mainly due to the backfat from wheat derived DDGS fed steers having lower levels of *trans*-18:1 and, consequently, lower levels of individual *trans*-18:1 isomers and a higher 11*t*-/10*t*- ratio compared to the backfat from corn derived DDGS steers (Aldai et al., 2010a).

Since there is limited information available on the effects of feeding wheat DDGS on beef quality and a lack of information regarding the effect of a wheat/corn DDGS, hereafter called blend DDGS, on meat quality, the objective of the present study was to compare a barley-based control finishing diet to the effects of including wheat, corn or blend DDGS at 40% of dietary DM on the quality and sensory attributes of meat derived from crossbred steers.

3.3 Materials and Methods

3.3.1 Animal Management and Diet Composition

This trial commenced in November 2008, at the University of Saskatchewan—Beef Cattle Research Station (Saskatoon, SK, Canada). In total, 288 commercial crossbred steers were purchased and subjected to a 70-day backgrounding period followed by a finishing period. The steers were implanted at the start of the test with Synovex S® (Pfizer Canada, Inc., Kirkland, QC) and re-implanted with Synovex Plus (Pfizer Canada, Inc., Kirkland, QC) at the start of the finishing period. The cattle were then randomly assigned to one of 24 pens and each pen was assigned to one of four dietary treatments. The control diet was a standard barley-based finishing diet comprised of 87.8% barley, 6.5% barley silage, and 5.7% supplement (DM basis). The other diets involved substitution of wheat, corn, or blend DDGS for barley grain in the control diet at 40% of the dietary DM. The wheat DDGS was supplied by Terra Grain Fuels (Belle Plaine, SK), the corn DDGS was purchased in two loads from ConAgra Foods (Omaha, NE) and from Blue Flint Ethanol (Underwood, ND). The blend DDGS was supplied by Husky Energy Inc. (Lloydminister, SK) and was the by-product of an ethanol fermentation run that used a 50:50 (w/w) wheat and corn grain mix. The chemical composition of the DDGS diets is reported in **Table 3-1**.

Table 3-1 Chemical composition of the wheat (n=2), corn (n=2) and blend (n=1) DDGS[1] (Obtained from Amat et al., 2011, unpublished data).

	DDGS Diet		
	Corn	**Wheat**	**Blend**
Dry Matter (%)	90.2	90.9	92.9
Crude Protein (%)	31.4	43.1	33.5
Neutral Detergent Fibre (%)	42.0	36.6	43.0
Acid Detergent Fibre (%)	12.3	14.8	11.3
Starch (%)	5.4	2.6	3.1
Crude Fat (%)	13.7	4.1	9.8
Ash (%)	4.7	5.9	5.4
Sulfur (%)	0.8	1.0	0.4

[1] DDGS = Dried Distillers Grains with Solubles.

From the 288 steers, eighty (twenty per treatment) were selected for in-depth meat quality analysis. Five steers from each of the four treatments were selected on the basis of live weight (625 kg target) on four separate slaughter dates. The selected steers at each slaughter date were loaded as a group (n = 20) at the University of Saskatchewan—Beef Cattle Research Station and transported (approximately 6 h) to Plains Processing Ltd. (PPL; Carman, MB, Canada). Upon receipt at PPL, the animals were held in lairage overnight with free access to water. The steers were slaughtered the following morning at the PPL abattoir, with all animals being maintained throughout the study under the guidelines established by the Canadian Council of Animal Care (1993).

3.3.2 Carcass Handling and Sample Collection

Following slaughter, the carcasses were split and chilled at 2°C (wind speed of 0.5 m/s) for 24 h. At 24 h, the left carcass sides were knife-ribbed at the Canadian grade site (between the 12^{th} and 13^{th} ribs) and exposed to atmospheric oxygen for twenty minutes. Following grading, carcass sides were quartered and loaded onto a refrigerated trailer for transport (approximately 20 min) to Keystone Processors Ltd. (KPL; Winnipeg, MB, Canada). Upon unloading at KPL, a back fat sample was removed from each carcass for subcutaneous fatty acid analysis in an area in close proximity to the grading location. Carcass quarters were refrigerated (4°C).

At 3 d post-mortem (PM), a six-bone rib section (7^{th}-12^{th} ribs) was removed from the left side of each carcass. The rib sections were labeled, vacuum-packaged, palletized,

and stored (4°C) at KPL until delivery to the University of Saskatchewan. Six days PM, the pallets were loaded onto a refrigerated trailer and transported (approximately 6 h) to the University of Saskatchewan. The rib sections were placed in cold storage (-1°C) until 13 d PM, when they were then transferred to the refrigerated meat processing room (<7°C) located in the University of Saskatchewan's Department of Food and Bioproduct Sciences Pilot Plant.

The *longissimus* muscle was isolated through removal of the fat cap and extraneous muscles and an initial pH reading was obtained by inserting a Hanna Electrode pH Meter (Hanna H2 9025 Microcomputer pH Meter; Hanna Instruments, Woonsocket, RI) into the *longissimus* muscle approximately 10.0 cm from the caudal end. Starting from the caudal end, one steak (2.5 cm thick) was removed for fatty acid analysis; three steaks (3.2 cm thick) were removed for sensory analysis and Warner-Bratzler shear force (WBSF) determination; one steak (2.0 cm thick) was removed for colour evaluation; and one steak (5 cm thick) was removed for thiobarbituric acid reactive substances (TBARS) and proximate analysis.

The steak for colour analysis was placed onto a Styrofoam tray with a soaker pad (Ultra Zap 1SW 1SPPERF Absorbent Soaker Pad; Paper Pak Industries, Washington, GA), over-wrapped with an oxygen permeable film (108 cc m^2 24 h^1 Huntsman choice wrap; Huntsman Packaging Corporation, Uniontown, OH) and placed in the meat processing room to bloom (3 h). The remaining steaks were vacuum-packaged and transferred back to cold storage.

Fourteen days PM, the steaks for TBARS and proximate analysis were ground twice (Hobart Grinder Model 4812 with a 3 mm grinder plate; The Hobart Manufacturing Co., Troy, OH). An 80 g sample of the grind was vacuum-packaged for subsequent TBARS analysis while the remaining grind was allocated for subsequent proximate analysis. *Longissimus* pH was measured on the freshly ground beef using the slurry pH method outlined in the *Handbook of Meat Analysis* (1985). In duplicate, 20 g of meat were placed in a small blender jar and diluted with 80 mL distilled water. The sample was blended (Osterizer 10 Speed Blender; Oster, Mexico City, Mexico) for 60 s and the pH was read by dispersing the electrode from a pH meter (Accumet Basic AB 15 Plus pH Meter; Fischer Scientific, Singapore) into the slurry. Approximately 4 g fresh meat was

also weighed, in duplicate, into pre-dried aluminum pans and analyzed for moisture content (Isotemp Oven Model 750F; Fischer Scientific, Singapore) using method 950.46 (AOAC, 1990). The remaining meat was vacuum-packaged; all of the vacuum-packaged meat was transferred to frozen storage (-30°C).

3.3.3 Objective and Subjective Colour Evaluation

Following blooming, objective colour measurements were made on the steak designated for colour analysis, in duplicate, from two surface locations (Hunter L* (lightness), a* (red-green axis), b* (yellow-blue axis); Commission Internationale de l'Eclairage, 1978) using the Hunter Lab Miniscan 45/0-L XE (Hunter Association Laboratory Inc., Reston, VA) equipped with Illuminant A and Observer 10 settings. The steaks were then placed in a retail display case (4°C; Hussmann SSM-6; Hussmann Corporation, Gloversville, NY) equipped with deluxe warm-white fluorescent lighting (F32T8/TL741 Fluorescent Bulb; Philips Inc., Somerset, NJ) adjusted to deliver an average of 1210 lx (Hunt et al., 1991) to assess retail storage life. Steaks were objectively evaluated, as previously described, every 24 h for 7 d. These steaks were also subjectively evaluated by five panelists on days 1, 2, 3, 4 and 7.

Panelists evaluated the steaks for retail appearance, lean colour score, percent surface discolouration, colour of discolouration, marbling score, and marbling colour using 8-point hedonic (1 = extremely undesirable and 8 = extremely desirable), 8-point descriptive (1 = white and 8 = extremely dark red), 7-point descriptive (1 = 0% and 7 = 100% discolouration), 7-point descriptive (1 = no browning and 7 = black), 6-point descriptive (1 = devoid and 6 = abundant), and 5-point descriptive (1 = white and 5 = brown) scales, respectively (Aldai et al. 2010b). Steaks were subsequently rotated daily to ensure exposure to all light intensities.

3.3.4 Proximate Analysis

The ground meat designated for proximate analysis was thawed (-1°C) overnight and ground (30 s) with the Osterizer blender. Crude fat content was analyzed by petroleum ether extraction (Labconco Goldfisch Apparatus; Labconco Corporation; Kansas City, MI) using method 960.39 (AOAC, 1990), nitrogen content was determined

by digestion (Buchi K435KB Digestion Unit; Buchi Labortechnik AG; Flawil, Switzerland) and distillation (Buchi B324 Distillation Unit; Buchi Labortechnik AG; Flawill, Switzerland) using method 981.10 (AOAC, 1990) and a nitrogen conversion factor of 6.25, and ash content was determined by heating in a muffle furnace (Isotemp 550-126 Muffle Furnace; Fischer Scientific, Dubuque; VI) using method 920.153 (AOAC, 1990).

3.3.5 TBARS Analysis

A subset of 48 animals (12 per treatment) was randomly selected for TBARS analysis. Meat ground for proximate analysis was divided into two portions: one for proximate analysis and one for raw TBARS analysis. Meat designated for raw TBARS analysis was then blended (30 s) with an Osterizer blender and placed into sterile sampling bags. The bags were stored under dark conditions (4°C) for 7 d. Raw TBARS analysis was conducted on only 36 animals (9 per treatment) because a set of TBARS samples had to be removed from the dataset due to problems encountered during analysis.

Meat for cooked TBARS analysis was thawed (4°C) for 24 h and cooked according to a method modified from Luciano et al. (2009). The vacuum-packaged samples were placed in a pre-heated water bath (75°C) and heated for 35 min, with a reference sample being monitored (Fluke 51 II Thermometer; Fluke Corporation; Everett, WA) to ensure an internal temperature of 70°C was obtained. Once cooked, the meat was transferred to an ice bath (15 min) and blended (30 s) with the Osterizer blender. The blended meat was placed into sterile sampling bags and stored under dark conditions (4°C) for 7 d.

TBARS analysis occurred on days 1, 3 and 7 according to a modified method of Witte et al. (1970) as reported by Bedinghaus and Ockerman (1995). A working solution was prepared by diluting a 0.02 M 1, 1, 3, 3-tetramethoxypropane (TMP) solution with distilled water to create a working solution (2.0×10^{-7} moles mL^{-1}). Standards were prepared by mixing 0.5 mL, 1.5 mL, 3.0 mL, and 4.5 mL working solution with a 50:50 solution of 20% trichloroacetic acid containing 1.6% phosphoric acid (TCA) and distilled water and diluting to 100 mL. Spiked samples were prepared, in duplicate, by adding 1.5 mL, 3.0 mL, and 4.5 mL of working solution to 5.00 ± 0.02 g control meat, followed by

the addition of 50 mL TCA and stomaching (2 min). An additional 45 mL distilled water was then added to the meat and stomached (1 min); the solution was filtered through Whatman #1 filter paper (Whatman International Ltd; Maidstone, England). Samples were prepared, in duplicate, by adding 50 mL TCA to 5.00 ± 0.02 g, stomaching (2 min), adding 50 mL distilled water, stomaching (1 min), and filtering through Whatman #1 filter paper. All samples were diluted to 100 mL using a 50:50 solution of TCA: distilled water before a 5.0 mL aliquot of filtrate was mixed with 5.0 mL 0.02M TBA reagent in a falcon tube (Falcon Blue Max 50 mL Polypropylene Conical Tube; Becton Dickinson Labware; Le Pont De Claix, France). The samples were placed in a boiling water bath (35 min) and cooled on ice (10 min). Absorbances were read against a blank at 532 nm through a quartz cuvette using Spectronic Genesys 5 Spectrophotometer (Milton Roy Company, Ivyland, PA). TBARS values (mg malondialdehyde per kg meat) were obtained by multiplying the absorbance of each sample by a constant (K) (Witte et al., 1970), whose formula was modified from Tarladgis et al. (1960), which takes into account the slope of the standard curve and the percent recovery of the system. A K-value of 21.31 was calculated from a slope of 1.63×10^7 and an 82.02% recovery of TMP.

3.3.6 Instrumental Shear Analysis

Steaks for Warner-Bratzler Shear Force (WBSF) analysis were allowed to thaw (4°C; 24 h) before a thawed weight was obtained. This weight was compared to the initial steak weight (obtained prior to freezing) to calculate drip loss. Oven-proof thermocouples were inserted into the geometric centre of the steak, and the steaks were grilled (Garland Grill ED30B; Condon Barr Food Equipment Ltd.; Edmonton, AB) to an internal temperature of 35.5°C, turned, and cooked to a final temperature of 71°C (Barnant 692-0000 Scanning Thermometer; Barnant Co.; Barrington, IL). Steaks were removed from the grill and cooled to an internal temperature of 50°C before being placed into polyethylene bags, sealed, and transferred to a 4°C cooler (24 h). The following day, six cores (2.5 cm x 1.2 cm x 1.2 cm) were removed parallel to the fibre grain. Peak shear force was determined on each core perpendicular to the fibre grain using a TMS Pro Texture Press equipped with a Warner-Bratzler shear head at a crosshead speed of 200 mm/min and 30 kg load cell using Texture Lab Pro v. 1.12 Software (Food Technology

Corporation; Sterling, VA), with the average peak shear force of each steak being reported.

3.3.7 Sensory Analysis

Sensory analysis was carried out at Agriculture and Agri-Food Canada (AAFC) - Lacombe Research Centre (LRC; Lacombe, AB) according to methods outlined in Aldai et al. (2010b). Prior to cooking, steaks were removed from refrigerated storage and raw weights were obtained. Steaks were then cooked to an internal temperature of 71°C by inserting a spear point temperature probe (10 cm) into the mid-point of the steak and grilling (Garland Grill ED30B; Condon Barr Food Equipment Ltd., Edmonton AB) to an internal temperature of 35.5°C, turning, and cooking to a final internal temperature of 71°C (Hewlett Packard HP34970 Data Logger; Hewlett Packard Co.; Boise ID). Steaks were cooled (5 min) before the final steak weight was obtained. Total cooking time was also recorded upon removal from the grill. Each steak was then cut into 1.3cm cubes, avoiding connective tissue and large fat pockets, with cubes from each sample being randomly assigned to a trained sensory panel. All panel evaluations were conducted in well-ventilated partitioned booths under red lighting (124 lx). Prior to evaluation, the cubes were placed in glass jars and allowed to equilibrate to an internal temperature of 71°C using a circulating water bath (Lindberg/Blue Model WB1120A-1; Kendro Laboratory Products; Asheville NC). Panellists were provided with distilled water and unsalted soda crackers to remove residual flavour notes between samples (Larmond, 1977). The attribute ratings were electronically recorded using Compusense 5, Release 4.6 Computer Software (Compusense Inc.; Guelph, ON) using a 9-point descriptive scale for all attributes, aside from flavour desirability and overall palatability, which was rated using an 9-point hedonic scale. Initial tenderness was rated on the first bite through the centre surface with the incisors (1 = extremely tough and 9 = extremely tender), while initial juiciness was rated after 3-5 chews with the molars (1 = extremely dry and 9 = extremely juicy). Beef flavour intensity (1 = extremely bland and 9 = extremely intense), off-flavour intensity (1 = extremely intense off-flavour and 9 = no off-flavour), amount of connective tissue (1 = abundant and 9 = none detected) and flavour desirability (1 = extremely undesirable and 9 = extremely desirable) were rated after 10 to 20 chews.

Overall tenderness (1 = extremely tough and 9 = extremely tender), sustainable juiciness (1 = extremely dry and 9 = extremely juicy), and overall palatability (1 = extremely undesirable and 9 = extremely desirable) was rated prior to expelling the meat cube. Each panellist also assigned one of the following flavours (metallic, off-sour, livery, grainy, bloody, other, unidentified, none) and textures (typical beef, mushy, mealy, spongy, rubbery) descriptors to each cube. Flavour and texture descriptors were reported as the percentage of panellists attributing that descriptor to that sample.

3.3.8 Fatty Acid Analysis

Fatty acid analysis was performed at AAFC-LRC. Methods for analysis were modified from Cruz-Hernandez et al. (2004) and Kramer et al. (2008). Backfat samples (50 mg) were freeze-dried and directly methylated with sodium methoxide. Intramuscular lipids were extracted from 1 g *longissimus* muscle using a 2:1 (v/v) mixture of chloroform: methanol (Kramer et al. 2008). Specifically, each *longissimus* sample was first homogenized (VirTis Cyclone IQ2 homogenizer; VirTis Company; Gardiner, NY) in 6 mL methanol (30 s at 15000 rpm) and then again with the addition of 12 mL chloroform. The mixture was filtered through a sintered glass funnel and the homogenizer was rinsed with an additional 12 mL chloroform. Another 6 mL methanol was added through the funnel, and the filtrate volume was adjusted to 25 mL using 1:1 chloroform: methanol. Next, 10 mL of 0.88% potassium chloride solution and 1 drop of 6 N hydrochloric acid were added, and the solution was mixed and centrifuged (5 min at 600 *g*) to separate the aqueous and organic phases. The bottom organic layer (chloroform) was removed and the 12 mL of chloroform used to rinse the homogenizer was filtered through the sintered glass funnel containing the meat residue and combined with the aqueous phase. The solution was, again, mixed and centrifuged to separate the phases and the bottom chloroform layer was collected and pooled with the first one. The chloroform was evaporated using a rotary evaporator (Bucho Model R-114; Buchi Labortechnik AG; Flawill, Switzerland) and total lipids were dissolved in 15 mL of chloroform. Lipid aliquots (10 mg) from each steak were separately methylated using acidic (methanolic HCl) and basic (sodium methoxide) reagents (Kramer et al., 2008). Fatty acid methyl esters (FAME) were analyzed using the GC and Ag^{+}-HPLC equipment

and methods outlined by Cruz-Hernandez et al. (2004), while the *trans*-18:1 isomers were analyzed using two complementary GC temperature programs (Kramer et al., 2008; Dugan et al., 2007).

3.3.9 Tocopherol Analysis

Tocopherol analysis was performed at AAFC-LRC. Approximately 30 g of ground meat was homogenized in a food processor (Robot Coupe Blixir BX3; Robot Coupe USA Inc., Ridgeland, MS) and the homogenate collected in Whirlpak bags (Nasco; Salida, CA) and stored in the freezer at -80°C until analysis. Samples were removed from the freezer and thawed overnight at 4°C prior to analysis. Tocopherol content was estimated by high performance liquid chromatography (HPLC; Alliance Waters, Separation module e2695 equipped with a multi-wavelength fluorescence detector 2475; Waters Corporation, Milford, MA) according to methods outlined by Hewavitharana et al. (2004). In summary, approximately 1 g of each sample was weighed, in duplicate, into 50 ml polyethylene screw top centrifuge tubes and capped to avoid oxidation of the samples during the process. Three hundred µl of internal standard (0.2 mg/ml α-tocopherol acetate) was added. The tubes were placed in ice and 4 ml of absolute ethanol was added, then homogenized. Five ml of reverse osmosis water was added to the tube and the contents homogenized for 15 seconds. Four ml aliquot of hexane-BHT was added and then homogenized for an additional 15 seconds. The samples were centrifuged (Avanti J-E Centrifuge, Beckman Coulter, Mississauga, ON) for 10 min. The top layer was transferred into an amber HPLC vial containing a 300 µl glass insert and, from each sample, 50 µl were analyzed for α-tocopherol content under fluorescence with an excitation wavelength at 295 nm.

3.3.10 Statistical Analysis

Data were analyzed using PROC MIXED option of SAS 9.2. Colour and TBARS data were analyzed using repeated measures, with diet and day as the main effects and day as the repeated measure using an ante-dependence covariance model. Subject effect was also taken into account by nesting diet within animal. The remaining data were analyzed as a one-way ANOVA, using diet as the main effect. Means were separated

using PDIFF procedure and Tukey-Kramer Highest Significant Difference. One animal (control) was eliminated from the statistical analysis as it was identified as a dark-cutter (pH of 6.31). Significance and trends were declared at $P \leq 0.05$ and 0.10, respectively.

3.4 Results and Discussion

3.4.1 Meat Quality

In general, few differences were found amongst dietary treatments in chemical composition, pH, drip loss, cook loss, cook time and WB shear force values **(Table 3-2)**. There were no differences amongst dietary treatments for fat and moisture content of the *longissimus* muscle, which is in accordance with Aldai et al. (2010b), Jenschke et al. (2008), and Shand et al. (1998), who also did not find differences in meat composition for animals finished on various distillers' grains diets.

Table 3-2 Effect of diet (treatment) on meat quality attributes of steers (n = 80).

	Treatments				SEM	*P*-value
		DDGS[1] Diet				
	Control	Corn	Wheat	Blend		
Moisture (%)	73.14	72.42	73.32	72.98	0.273	0.119
Protein (%)	22.33	22.56	22.07	22.35	0.153	0.169
Fat (%)	3.11	3.62	3.32	3.45	0.298	0.673
Ash (%)	1.11	1.14	1.11	1.12	0.011	0.182
pH[2]	5.55	5.50	5.51	5.51	0.017	0.140
Drip loss (%)	0.96	1.06	1.10	1.01	0.058	0.339
Cook loss (%)	25.45	25.93	25.39	24.84	0.805	0.819
Cook time (sec/g)	5.24	5.23	5.16	5.34	0.186	0.929
WBSF (N)	71.67	80.14	74.28	71.12	4.03	0.377

[1] DDGS = Dried Distillers Grains with Solubles.

[2] Dark cutter removed from control group prior to analysis.

As with chemical composition, no differences amongst dietary treatments were observed for pH and drip loss. Again, this is in contrast to Aldai et al. (2010b), who found steaks from steers fed a barley control diet to have a slightly lower drip loss compared to steaks from steers fed wheat and corn DDGS. This was attributed to the control steaks having a numerically higher ultimate pH value (Aldai et al., 2010b), which relates to changes in the structure of myofibrillar proteins and their capacity to hold water

within the meat (Hamm, 1975). Likewise, Koger et al. (2010) observed the ultimate muscle pH values of the *longissimus* muscle were higher in steers fed DDGS when compared to those fed wet distillers' grains plus solubles (WDGS). A post-mortem decrease in ultimate pH results from the production of lactic acid from glycogen (Lawrie, 1998); therefore, the ultimate muscle pH is dependent on the amount of muscle glycogen present at the time of slaughter (Koger et al., 2010).

According to Koger et al. (2010), glycogen levels are influenced by the energy concentration in the diet of the animal in the month prior to slaughter, with Immonen et al. (2000) reporting that high-energy diets increased glycogen stores and decreased the ultimate pH when compared with low-energy, roughage diets. Due to the removal of starch from the grain stock during fermentation, it was hypothesized that feeding finishing steers DDGS would lower the glycolytic potential (Koger et al., 2010). Differences in glycolytic potential could explain why Aldai et al. (2010b) experienced a numerically higher ultimate pH in steaks obtained from cattle fed a barley-based finishing diet when compared to cattle finished on a DDGS diet, although no differences were observed in the present study.

As previously indicated, no dietary differences were observed for WB shear force values. The results of studies conducted by Smith et al. (1977) and Coleman et al. (1995) indicate that tenderness, as measured by WBSF, was sensitive to dietary effects; however, the present results are in agreement with Aldai et al. (2010b), who also did not observe any differences in shear force following 48 h and 20 d aging for beef steers fed varying levels of corn or wheat DDGS. It is possible that dietary differences were not detected in the present study due to a lack of power in the data as a result of a small sample size. Although the number of animals analyzed in this study was similar to Aldai et al. (2010b) and Koger et al. (2010), mathematical calculations show that the current WBSF experiment only had 12% explanatory power. The power of the experiment could be increased by increasing the sample size; however, financial constraints limited the number of animals available for analysis. Other researchers also found that feeding DGS did not affect instrumental tenderness, as measured by WBSF (Koger et al., 2010; Leupp et al., 2009). Using a trained sensory panel, Gordon et al. (2002) did note improvements in overall tenderness of ribeye steaks from heifers as dietary inclusion of DDGS

increased up to 75%; however, Roeber et al. (2005) reported no differences in shear values of steaks obtained from steers fed increasing levels of DDG in whole corn-based finishing diets.

With these findings in mind, it can be concluded that feeding steers a DDGS diet consisting of wheat and/or corn DDGS does not have an effect on meat composition, pH, drip loss, cook loss, cook time, or WB shear force values when compared to steers fed a barley control diet, despite compositional differences in the diet.

3.4.2 Fatty Acid Composition

3.4.2.1 Dietary Fatty Acids

In accordance with Dugan et al. (2010), substitution of wheat DDGS for barley increased the amount of dietary crude fat from 2.23 to 3.78% **(Table 3-3)**. As expected, substitution of corn DDGS and the wheat/corn DDGS for barley resulted in elevated levels of crude fat above those found in the wheat DDGS diet, which is due to corn containing approximately twice the amount of oil than wheat. The fatty acid composition of the control diet was consistent with previous reports for barley-based finishing diets (Dugan et al., 2010); however, it did differ from Aldai et al. (2010a) in regards to two fatty acids. Specifically, the barley diet in the current study contained higher levels of 16:0 and lower levels of 18:3n-3 than the barley diet used by Aldai et al. (2010a) (differences of 21.4 to 18.5% and 4.60 to 7.03%, respectively).

Table 3-3 Crude fat and fatty acid composition of experimental diets.

Feed composition	Treatment [1]			
		DDGS Diet		
	Control	Corn	Wheat	Blend
Crude fat, % (DM basis)	2.23	5.97	3.78	5.06
Fatty acid , % of total fatty acids				
∑ SFA	24.82	18.75	21.94	20.30
14:0	0.35	0.13	0.22	0.15
16:0	21.41	15.59	19.13	17.25
17:0	0.12	0.08	0.12	0.11
18:0	1.80	1.99	1.62	1.89
20:0	0.36	0.42	0.27	0.37
22:0	0.38	0.23	0.28	0.25
24:0	0.40	0.31	0.30	0.28
∑ MUFA	21.53	25.97	18.73	23.57
9*c*-16:1[2]	0.17	0.14	0.16	0.14
9*c*-18:1	19.38	24.54	16.87	21.95
11*c*-18:1	1.19	0.91	1.04	0.98
11*c*-20:1	0.79	0.38	0.66	0.50
∑ PUFA	53.66	55.28	59.32	56.13
18:2n-6	49.06	52.71	54.83	52.83
18:3n-3	4.60	2.57	4.49	3.30

[1] Per 100g of total mixed ration (TMR) on a dry matter (DM) basis; n=2 for each TMR.

[2] *c = cis.*

On the whole, the fatty acid composition of the diets differed in regards to four different fatty acids: 16:0, 9*c*-18:1, 18:2n-6 and 18:3n-3. Of these fatty acids, linoleic (18:2n-6) and α-linolenic (18:3n-2) acid are amongst the most important when considering the fatty acid profile of the resulting meat. Conjugated linoleic acid (CLA) is produced from the partial biohydrogenation of linoleic acid and α-linolenic acid in the rumen by ruminal microorganisms (Lourenco et al., 2010). Based on the fatty acid profiles of the dietary treatments, it can be hypothesized that feeding a DDGS diet will produce adipose tissue with elevated levels of CLA.

3.4.2.2 Subcutaneous Fatty Acids

The fatty acid composition of beef is heavily influenced by rumen microflora because the rumen bacteria isomerize and hydrogenate dietary polyunsaturated fatty acids (PUFA), resulting in the accumulation of metabolic intermediates (Aldai et al., 2010a). Compared to wheat DDGS, corn DDGS has a higher oil content (Rasco et al., 1987). When examining the fatty acid profiles of wheat and corn oils, both are known to be rich in 18:2n-6 (Becker, 2008); therefore, this would provide additional substrate for ruminal biohydrogenation (Aldai et al., 2010a).

Replacing barley grain with corn and blend DDGS resulted in lower ($P<0.001$) concentrations of branched-chain fatty acids (BCFA) in the back fat of beef steers **(Table 3-4)**. *Iso*- and *anteiso*- fatty acids are unique biohydrogenation products in that they contain a methyl substituent on the penultimate (n-1) (*iso*-) or the antepenultimate (n-2) (*anteiso*) carbon of an otherwise straight chain alkyl chain (Hauff and Vetter, 2010). These BCFA are commonly distributed in the environment and other foodstuffs, although their concentrations in foods are generally low, ranging from only 1-3% of the total lipid content, and are typically stored in the nonpolar triacylglycerols (Hauff and Vetter, 2010). However, these BCFA are characterized as anticancer agents against human tumor cells in a manner similar to conjugated linoleic acid (Wongtangtintharn et al., 2004). BCFA induce apoptotic cell death and inhibit *in vivo* growth of human cancer cells (Yang et al., 2000) by affecting the cell replication process through modulation of fatty acid metabolism (Wongtangtintharn et al., 2004).

Table 3-4 Percent saturated fatty acid composition of back fat from steers fed various DDGS diets.

Fatty acid, [1] wt %	Treatment				SEM	*P*-value
		DDGS Diet				
	Control	Corn	Wheat	Blend		
∑ BCFA	1.74[a]	1.42[b]	1.74[a]	1.51[b]	0.052	<0.001
15:0iso	0.126	0.119	0.145	0.121	0.008	0.077
15:0ai	0.205	0.184	0.209	0.188	0.012	0.384
16:0iso	0.192[a]	0.171[b]	0.221[a]	0.171[b]	0.008	<0.001
17:0ai	0.713[a]	0.568[b]	0.682[a]	0.609[b]	0.018	<0.001
17:0iso	0.333[a]	0.261[b]	0.323[a]	0.292[ab]	0.011	<0.001
18:0iso	0.167[a]	0.118[b]	0.164[a]	0.127[b]	0.007	<0.001
∑ SFA	46.22[a]	43.65[b]	43.93[ab]	44.21[ab]	0.630	0.021
14:0	3.85[a]	3.60[ab]	3.34[b]	3.30[b]	0.137	0.020
15:0	0.752[a]	0.477[b]	0.542[b]	0.501[b]	0.038	<0.001
16:0	26.13[a]	23.34[c]	24.71[b]	23.52[bc]	0.365	<0.001
17:0	1.64[a]	1.00[b]	1.05[b]	1.07[b]	0.072	<0.001
18:0	11.86[b]	13.56[ab]	12.29[ab]	14.01[a]	0.515	0.012
19:0	0.069[ab]	0.065[b]	0.079[a]	0.078[ab]	0.003	0.014
20:0	0.110[ab]	0.115[ab]	0.107[b]	0.127[a]	0.005	0.017
22:0	0.057[b]	0.060[ab]	0.060[ab]	0.073[a]	0.004	0.031

[1] Fatty acids <0.05% of total fatty acids not reported. BCFA: branched-chain fatty acids (includes *iso* and *anteiso*): ∑ BCFA = *iso*- 15:0 + *anteiso*- 15:0 + *iso*- 16:0 + *anteiso*- 17:0 + *iso*- 18:0; ∑ SFA = ∑ BCFA + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 19:0 + 20:0 + 21:0 + 22:0.

The lower levels of BCFA in the back fat of steers fed corn or blend DDGS was mainly due to lower concentrations of *iso*-16:0, *anteiso*-17:0 and *iso*-18:0. This is important because it was observed that the highest anti-tumor activity was observed with *iso*-16:0, with this activity decreasing with an increase or decrease in the chain-length away from 16:0 (Wongtangtintharn et al., 2004). Similar results were obtained by Aldai et al. (2010a), who found back fat from steers fed a control barley finishing diet to have the highest BCFA content, with increasing levels of either corn or wheat DDGS leading to linear reductions in BCFA. This was attributed to lower ruminal propionate production in these diets as a result of lower levels of readily fermentable dietary barley starch (Hristov et al., 2002). Unlike Aldai et al. (2010a), no differences in BCFA were observed in back fat obtained from steers fed the control and wheat DDGS diets in the present study. Although it is not clear why differences in BCFA concentrations are observed, it is

possible that these differences result from differences in ruminal propionate production or from differences in branched-chain amino acid degradation.

As was observed with BCFA, feeding steers a corn DDGS finishing diet resulted in lower ($P<0.05$) levels of saturated fatty acids (SFA) in the back fat when compared to feeding steers a barley diet, while no differences were observed for wheat and blend DDGS **(Table 3-4)**. These findings are in contrast to Gill et al. (2008), who found no differences in SFA content in striploins obtained from steers who were fed steam-flaked corn versus diets containing either 15% corn/sorghum dried/wet distillers' grains (DM basis). These findings are also in contrast to Aldai et al. (2010a) and Depenbusch et al. (2009), who also did not find any differences in the SFA contents between cattle fed various dietary levels of DDGS. This reduction in SFA in steers fed corn DDGS is most likely caused by a reduction in 16:0 due to lower *de novo* synthesis (Palmquist, 1996), which could either be related to the substrate supply from starch fermentation or to the inhibition of SFA synthesis by polyunsaturated fatty acids or their biohydrogenation products. Although differences were detected in total SFA content between steers fed the control versus corn DDGS in the present study, there were no differences in total SFA content between steers fed the DDGS diets.

In other research examining SFA in beef animals, Koger et al. (2004) noted an increase in stearic (18:0) acid and a decrease in margaric (17:0) acid when distillers' grains were added to finishing diets, while Gill et al. (2008) found greater proportions of margaric and stearic acids in striploin steaks from steers fed distillers' grains than from those fed a steam-flaked corn diet. Like Koger et al. (2004), the present study found that feeding finishing steers a DDGS diet resulted in decreased ($P<0.001$) concentrations of margaric acid and increased ($P<0.05$) concentrations of stearic acid in the back fat when compared to feeding a barley finishing diet. This increase in stearic acid is related to more complete biohydrogenation of PUFA in the DDGS diets (Lourenco et al., 2010). Increased concentrations of margaric or stearic acids are not a major concern because they do not aid in increasing human plasma cholesterol concentrations (Gill et al., 2008). Although beef saturated fat contains approximately 40% stearic acid (Baghurst, 2004), it is lauric (12:0), myristic (14:0) and palmitic (16:0) acids that are primarily responsible for increasing plasma low-density lipoprotein and total cholesterol concentrations in the

human body (Hegsted et al., 1965). Consequently, this study found that feeding finishing steers wheat and blend DDGS resulted in decreased (P<0.05) levels of myristic acid and feeding steers a DDGS diet resulted in decreased (P<0.001) levels of palmitic acid when compared to feeding steers the control diet.

Unlike SFA, no dietary differences were observed for total monounsaturated fatty acids **(Table 3-5)**. These findings are supported by Dugan et al. (2010), Depenbusch et al. (2009), Gill et al. (2008) and Koger et al. (2004), who all reported no differences in MUFA concentrations for steers fed various distillers' grains diets. Although the total MUFA concentrations were not different among diets, there were some significant differences in the *cis*- and *trans*- MUFA profile in the back fat of steers fed corn DDGS versus the other diets. Total *cis*-MUFA were lowest (P<0.05) in corn DDGS fed steers and highest in control fed steers, with the diets containing wheat DDGS displaying intermediate values. These findings are in accordance with Aldai et al. (2010a), who also observed the *cis*-MUFA content to be highest in the back fat tissue of steers fed a control diet and lowest in the back fat tissue of steers fed corn DDGS.

When rumen bacteria isomerize and hydrogenate dietary polyunsaturated fatty acids (PUFA), metabolic intermediates can accumulate (Aldai et al., 2010a); rumenic acid (9*c*,11*t*-18:2) and its precursor vaccenic acid (11*t*-18:1), which is the common intermediate produced from the biohydrogenation of linoleic acid (18:2) (Gill et al., 2008), have been shown to demonstrate known health benefits in animal models when consumed (Collomb et al., 2006; Belury, 2002). In contrast, 10*t*-18:1 has been shown to have a negative impact on plasma cholesterol concentrations in animal models (Bauchart et al., 2007) and with coronary heart disease in humans (Hodgson et al., 1996). The *trans*-18:1 isomer profile in beef can be influenced by diet, with diets high in rapidly fermenting carbohydrates producing high levels of 10*t*-18:1 (Dugan et al., 2007; Hristov et al., 2005), while cattle on forage have been shown to increase vaccenic acid production relative to 10*t*-18:1 (Aldai et al., 2008). Therefore, finishing cattle on DDGS in place of ground barley would lower dietary starch levels, subsequently increasing PUFA levels available for biohydrogenation, while increasing the crude fibre levels, which may elevate rumen pH and favour the production of 11*t*-18:1.

Table 3-5 Percent unsaturated fatty acid composition of back fat from steers fed various DDGS diets.

Fatty acid, [1] wt %	Treatment				SEM	*P*-value
		DDGS Diet				
	Control	Corn	Wheat	Blend		
∑ MUFA	49.59	48.85	48.44	48.72	0.656	0.643
∑ *cis* MUFA	47.40^{a}	44.27^{b}	46.20^{ab}	46.51^{ab}	0.730	0.026
∑ *trans* MUFA	2.19^{b}	4.59^{a}	2.24^{b}	2.21^{b}	0.274	<0.001
6*t*/7*t*/8*t*-18:1	0.175^{c}	0.444^{a}	0.245^{bc}	0.268^{b}	0.023	<0.001
9*t*-18:1	0.109^{b}	0.308^{a}	0.124^{b}	0.116^{b}	0.025	<0.001
10*t*-18:1	1.01^{b}	2.10^{a}	0.681^{b}	0.629^{b}	0.204	<0.001
11*t*-18:1	0.229^{b}	0.687^{a}	0.300^{b}	0.308^{b}	0.033	<0.001
12*t*-18:1	0.153^{c}	0.318^{a}	0.224^{b}	0.251^{b}	0.011	<0.001
13*t*/14*t*-18:1	0.328^{c}	0.507^{a}	0.441^{ab}	0.434^{b}	0.019	<0.001
15*t*-18:1	0.162	0.211	0.224	0.213	0.019	0.113
16*t*-18:1	0.093^{b}	0.119^{ab}	0.125^{ab}	0.135^{a}	0.009	0.009
11*t*/10*t*-18:1 ratio	0.300^{b}	0.426^{ab}	0.481^{ab}	0.601^{a}	0.051	<0.001
∑ n-3 PUFA	0.388^{c}	0.445^{b}	0.578^{a}	0.499^{b}	0.015	<0.001
18:3n-3	0.260^{c}	0.307^{bc}	0.441^{ab}	0.348^{b}	0.014	<0.001
22:5n-3	0.077	0.084	0.080	0.087	0.005	0.623
∑ n-6 PUFA	2.37^{b}	5.35^{a}	5.47^{a}	5.09^{a}	0.204	<0.001
18:2n-6	2.01^{b}	4.95^{a}	4.99^{a}	4.67^{a}	0.200	<0.001
18:3n-6	0.052	0.042	0.058	0.054	0.004	0.058
20:2n-6	0.076^{c}	0.095^{b}	0.110^{a}	0.094^{b}	0.004	<0.001
20:3n-6	0.089^{c}	0.121^{b}	0.148^{a}	0.117^{b}	0.007	<0.001
20:4n-6	0.068	0.067	0.075	0.074	0.004	0.449
22:4n-6	0.073	0.071	0.085	0.086	0.006	0.205
∑ PUFA	2.76^{b}	5.79^{a}	6.05^{a}	5.59^{a}	0.214	<0.001
n-6:n-3	6.02^{c}	12.07^{a}	9.52^{b}	10.15^{b}	0.330	<0.001

[1] Fatty acids <0.05% of total fatty acids not reported. MUFA: monounsaturated fatty acids: ∑ MUFA = 9*c*-14:1 + 9*c*-15:1 + 7*c*-16:1 + 9*c*-16:1 + 9*c*-17:1 + 9*c*-18:1 + 11*c*-18:1 + 12*c*-18:1 + 13*c*-18:1 + 14*c*-18:1 + 15*c*-18:1 + 9*c*-20:1 + 11*c*-20:1 + 6*t*/7*t*/8*t*-18:1 + 9*t*-18:1 + 10*t*-18:1 + 11*t*-18:1 + 12*t*-18:1 + 13*t*-14*t*-18:1 + 15*t*-18:1 + 16*t*-18:1; ∑ cis MUFA = 9*c*-14:1 + 9*c*-15:1 + 7*c*-16:1 + 9*c*-16:1 + 9*c*-17:1 + 9*c*-18:1 + 11*c*-18:1 + 12*c*-18:1 + 13*c*-18:1 + 14*c*-18:1 + 15*c*-18:1 + 9*c*-20:1 + 11*c*-20:1; ∑ trans MUFA = 6*t*/7*t*/8*t*-18:1 + 9*t*-18:1 + 10*t*-18:1 + 11*t*-18:1 + 12*t*-18:1 + 13*t*/14*t*-18:1 + 15*t*-18:1+ 16*t*-18:1. PUFA = polyunsaturated fatty acids: ∑ n-3 PUFA = 18:3n-3 + 20:3n-3 + 22:5n-3; ∑ n-6 PUFA = 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6; ∑ PUFA = n-3 PUFA + n-6 PUFA; *c* = *cis*; *t* = *trans*.

Increases in the total *trans*-MUFA have been observed in duodenal digesta (Vander Pol et al., 2009) and in strip loin steaks when feeding DDGS derived from corn, with these differences possibly being related to their greater oil content (Dugan et al., 2010). Likewise, feeding corn DDGS to steers resulted in the highest (P<0.001) total *trans*-MUFA content, as well as higher levels of individual *trans*-18:1 (6*t*/7*t*/8*t*-, 9*t*-, 10*t*-, 11*t*-, 12*t*-, 13*t*/14*t*-18:1) when compared to the back fat from steers fed other dietary treatments **(Table 3-5)**, which is in accordance with the findings of Aldai et al. (2010a), who also observed that feeding increasing levels of corn DDGS resulted in a linear increase in total *trans*- fatty acids. Likewise, Dugan et al. (2010) observed that feeding increasing amounts of wheat DDGS led to a linear reduction 10*t*-18:1 and linear increases in several other *trans*-18:1 isomers, which included 11*t*-18:1, in the brisket fat composition of British crossbred heifers. It was also observed that the *trans*-18:1 composition in the back fat of steers fed blend DDGS was very similar to that of steers fed wheat DDGS, meaning that some corn DDGS can be added to a diet with wheat DDGS without drastically increasing the levels of *trans* fats with negative health effects.

However, the relative flow of PUFA through the major biohydrogenation pathways can be evaluated by the 11*t*-/10*t*-18:1 ratio, with a higher ratio denoting improvements in its healthfulness to consumers (Aldai et al., 2010a). In this regard, feeding steers a blend DDGS diet resulted in a higher (P<0.001) (more desirable) ratio, while feeding steers a control diet resulted in the lowest ratio, although the 11*t*-/10*t*-18:1 ratio was still below one for all dietary treatments. These ratios are in accordance with Aldai et al. (2010a), who found that feeding steers wheat DDGS resulted in the most desirable ratio in the back fat; however, the ratio contrasts to values of over 6 that occur in cattle fed diets containing high levels of forage (Aldai et al., 2008).

Unlike *trans*-18:1 fatty acids, the levels of PUFA were consistent with the levels of DDGS inclusion in the diet, with steers being fed a DDGS diet having higher (P<0.001) PUFA levels than control steers **(Table 3-5)**. The n-6 and n-3 fatty acids are commonly associated with beneficial health effects on human health, with linoleic acid being needed to synthesize pro-inflammatory eicosanoids and increasing linoleic acid content in human diets being found to lower blood cholesterol concentrations and reduce the risk of coronary heart disease, while n-3 fatty acids moderate inflammation by

competing with n-6 fatty acids (Gill et al., 2008). However, the prevention and treatment of coronary heart disease in humans has also been linked with the consumption of eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) (Depenbusch et al., 2009).

Schingoethe et al. (1999) determined that a 31.2% inclusion of corn WDG to a corn silage-based diet provided an additional 2% fatty acids to the diet, which was mainly due to increases in 18:2 and 18:3 PUFA. These elevated levels were confirmed by Dugan et al. (2010), who found that feeding increasing levels of wheat DDGS subsequently led to a linear increase in 18:2n-6 and total n-6 fatty acids in brisket fat. However, it is believed that in order for these dietary fats to be beneficial to human health, diets need to contain a proper ratio of n-6 to n-3 fatty acids (5:1; World Health Organization, 2003). The values for the n-6:n-3 ratios are in accordance with Aldai et al. (2010a), who found that feeding corn DDGS resulted in the highest ratios and feeding the control resulted in the lowest ratio, with wheat DDGS being intermediate. Likewise, feeding corn DDGS resulted in the highest ($P<0.001$) ratio, while feeding wheat and blend DDGS resulted in intermediate ratios, and feeding the control resulted in the lowest ratio. The high ratios found in this study are largely the result of feeding high concentrate diets because beef muscle in steers fed a concentrate diet have greater concentrations of n-6 fatty acids (Enser et al., 1998). Therefore, improving the n-6:n-3 ratio in feedlot cattle can be difficult simply because grain diets are naturally high in n-6 fatty acids (Gill et al., 2008).

Similar to PUFA concentrations, Dugan et al. (2010) found that feeding increasing amounts of wheat DDGS led to a trend for a linear increase in total conjugated linoleic acid (CLA), which was mainly attributed to a linear increase in 9*c*,11*t*-18:2, in the brisket fat of feedlot heifers. Similar results were obtained by Depenbusch et al. (2009), who found that concentrations of CLA linearly increased in the intramuscular fat of cooked *longissimus* steaks as dietary levels of corn DDGS increased from 0 to 75%. CLA is a product of ruminal biohydrogenation of polyunsaturated fatty acids (Kelly et al., 1998) and has been shown to protect against cancer, inflammation and diabetes in experimental animal models (Belury, 2002; Pariza et al., 2001), while some recent studies have suggested that it reduces the risk of breast and colorectal cancers (Larsson et al.

2005; Aro et al. 2000) and acute myocardial infarctions in humans (Warensjo et al., 2004).

As previously mentioned, rumenic acid (9*c*,11*t*-18:2), which is derived from vaccenic acid (11*t*-18:1), has been demonstrated to have health benefits (Collomb et al., 2006). Total rumenic acid concentrations were highest ($P<0.001$) in the back fat obtained from steers fed corn DDGS and lowest in the back fat of steers fed the control diet, with steers fed a diet containing DDGS fermented from wheat displaying intermediate values **(Table 3-6)**. The greater quantity of 9*c*,11*t*-18:2 can be attributed to the greater content of 11*t*-18:1 due to the fact that most 9*c*,11*t*-18:2 is known to be derived from endogenous desaturation of 11*t*-18:1 by Δ^9-desaturase (Griinari et al., 2000). DDGS derived from the fermentation of wheat and corn also resulted in a reduction of 9*t*,11*c*-18:2, which is related to concentrate feeding; having a negative image for human health (Kramer et al., 2004). Feeding a DDGS diet also results in increased concentrations of 10*t*,12*c*-18:2, which has the potential for beneficial human health because it has been shown to hinder obesity by inhibiting lipogenesis (McGuire and McGuire, 2000). However, recent work by Kennedy et al. (2011) has shown 10*t*,12*c*-18:2 to increase inflammation and insulin resistance in human adipocytes by increasing calcium levels. It is important to note that, although changes in the 10*t*,12c-18:2 isomer were statistically significant, overall concentration differences are negligible.

Table 3-6 Percent conjugated linoleic acid (CLA) composition of back fat from steers fed various DDGS diets.

Fatty acid, wt %	Treatment				SEM	*P*-value
		DDGS Diet				
	Control	Corn	Wheat	Blend		
∑ CLA	0.561^{c}	0.872^{a}	0.669^{bc}	0.687^{b}	0.030	<0.001
7*t*,9*c*-18:2	0.089^{b}	0.177^{a}	0.108^{b}	0.114^{b}	0.009	<0.001
9*c*,11*t*-18:2	0.306^{b}	0.480^{a}	0.384^{ab}	0.397^{ab}	0.026	<0.001
9*t*,11*c*-18:2	0.072^{ab}	0.077^{a}	0.063^{ab}	0.060^{b}	0.004	0.011
10*t*,12*c*-18:2	0.021^{c}	0.054^{a}	0.036^{b}	0.041^{b}	0.002	<0.001
11*t*,13*c*-18:2	0.008	0.008	0.007	0.008	0.000	0.83
11*c*,13*t*-18:2	0.012	0.012	0.011	0.011	0.001	0.663
8*t*,10*c*-18:2	0.011	0.013	0.011	0.011	0.001	0.052

∑ CLA = 9*t*,11*c*-18:2 + 9*c*,11*t*-18:2 + 7*t*,9*c*-18:2 + 10*t*,12*c*-18:2 + 11*t*,13*c*-18:2 + 11*c*,13*t*-18:2 + 8*t*,10*c*-18:2 + (*c*,*t*-*t*,*c*)12,14-18:2 + 12*t*,14*t*-18:2 + 11*t*,13*t*-18:2 + 10*t*,12*t*-18:2 + 9*t*,11*t*-18:2.

In the current study, similarities were also observed in the back fat of steers fed wheat and blend DDGS in regards to PUFA biohydrogenation products. It is possible that the fermentation of wheat in the blend DDGS diet might support the production of vaccenic acid producing bacteria as opposed to 10*t*-18:1 producing bacteria. Likewise, the higher oil level in the corn DDGS might influence the production of 10*t*-18:1, as was shown by Duckett et al. (2002) who found diets containing higher levels of corn oil may alter the microbial populations that favour the *trans*-10 pathway of linoleic acid biohydrogenation. Lastly, it is possible that the higher oil levels found in corn DDGS may be inhibiting the hydrogenation from *trans*-18:1 to 18:1 (Lourenco et al., 2010).

3.4.2.3 Intramuscular Fatty Acids

When comparing the fatty acid compositions between the subcutaneous and intramuscular fats, marked differences were observed. The striking differences between back fat and intramuscular fat relate to the neutral to phospholipid ratio. Because there is a higher concentration of phospholipids in the intramuscular fat, there are more PUFA present. Therefore, differences in fatty acid levels can be partially attributed to a dilution by PUFA.

As with the crude fat analysis, no differences were observed in the total *longissimus* fatty acid content, on a milligram per gram basis. Unlike in the back fat of steers, feeding barley grain, corn DDGS and blend DDGS resulted in lower ($P<0.001$) concentrations of BCFA in the intramuscular fat **(Table 3-7)** compared to steers fed wheat DDGS. These findings are in direct contrast to Aldai et al. (2010a), who observed that the back fat of steers fed a barley control diet had the highest BCFA content and that increasing levels of either corn or wheat DDGS led to a linear reduction in BCFA. These lower levels of BCFA in the DDGS diets were attributed to lower dietary levels or readily fermentable barley starch, resulting in lower ruminal propionate production (Hristov et al., 2002). In the current study, it is possible that there was a shift in production streams, with more BCFA coming from branched-chain amino acids. This is possible because BCFA originate from microbial matter in the rumen so feeding unprotected lipids to ruminants can induce changes in the rumen microbial population, resulting in an alteration to the outflow of these products (Rego et al., 2009). In terms of BCFA

research, there is little information available regarding the composition in meat products, mostly due to the fact that there are no suitable methods present for the routine analysis of BCFA (Hauff and Vetter, 2010). Therefore, it is difficult to determine why BCFA concentrations differed from the subcutaneous to the intramuscular fat and why concentrations differed from Aldai et al. (2010a) to this study; however, it can be assumed that some differences can be attributed to the dilution of PUFA in *longissimus* muscle.

Table 3-7 Percent saturated fatty acid composition of intramuscular fat from steers fed various DDGS diets.

	Treatment				SEM	*P*-value
		DDGS Diet				
Item [1]	Control	Corn	Wheat	Blend		
∑ Fatty Acids, mg/g	33.96	35.83	32.75	35.38	2.68	0.843
Fatty acid, wt %						
∑ BCFA	1.36^{b}	1.19^{b}	1.43^{a}	1.21^{b}	0.035	<0.001
15:0iso	0.118^{ab}	0.098^{b}	0.123^{a}	0.106^{ab}	0.006	0.028
15:0ai	0.153	0.148	0.169	0.146	0.009	0.238
16:0iso	0.126^{ab}	0.112^{b}	0.144^{a}	0.113^{b}	0.006	0.002
17:0ai	0.521^{a}	0.447^{b}	0.523^{a}	0.470^{b}	0.012	<0.001
17:0iso	0.300^{ab}	0.272^{b}	0.333^{a}	0.263^{b}	0.014	0.003
18:0iso	0.139^{a}	0.110^{bc}	0.135^{ab}	0.109^{c}	0.007	0.002
∑ SFA	42.77^{a}	41.31^{ab}	40.93^{b}	41.28^{b}	0.398	0.008
C14:0	2.62^{a}	2.57^{ab}	2.25^{b}	2.39^{ab}	0.093	0.022
C15:0	0.595^{a}	0.413^{b}	0.433^{b}	0.420^{b}	0.033	<0.001
C16:0	24.64^{a}	22.84^{b}	22.89^{b}	22.78^{b}	0.31	<0.001
C17:0	1.56^{a}	0.980^{b}	1.09^{b}	1.03^{b}	0.074	<0.001
C18:0	13.02^{b}	14.16^{ab}	13.86^{ab}	14.32^{a}	0.327	0.031
C19:0	0.081	0.076	0.084	0.083	0.005	0.698
C20:0	0.128^{b}	0.135^{ab}	0.171^{a}	0.136^{ab}	0.01	0.014
C22:0	0.101	0.109	0.131	0.104	0.012	0.278
∑ DMA	1.37	1.45	1.67	1.56	0.115	0.296
16:0DMA	0.825	0.765	0.895	0.815	0.065	0.562
16:1DMA	0.202	0.241	0.265	0.266	0.018	0.051
18:0DMA	0.480^{b}	0.347^{ab}	0.446^{a}	0.510^{ab}	0.038	0.020

[1] Fatty acids <0.05% of total fatty acids not reported. BCFA: branched-chain fatty acids (includes *iso* and *anteiso*): ∑ BCFA = *iso*- 15:0 + *anteiso*- 15:0 + *iso*- 16:0 + *anteiso*- 17:0 + *iso*- 18:0. ∑ SFA = ∑ BCFA + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 19:0 + 20:0 + 21:0 + 22:0. DMA: dimethyl acetals: ∑ DMA = 16:0DMA + 16:1DMA + 18:0DMA.

In terms of total SFA, steers fed a diet containing DDGS fermented from wheat had lower (P<0.005) levels of SFA in the intramuscular fat than steers fed the barley control diet. Again, total levels of SFA were lower in the intramuscular fat than in the subcutaneous fat due to an increase in total PUFA concentrations, which doubled in comparison to the PUFA contents in the subcutaneous fat **(Table 3-7)**. Total SFA contents from the intramuscular fat of steers fed a diet containing DDGS fermented from wheat were lower (P<0.05) than the total SFA contents of steers fed the barley control diet. This differs from subcutaneous fat, where the back fat obtained from steers fed corn DDGS had lower concentrations of SFA than steers fed the barley control diet. Differences between subcutaneous and intramuscular fats can be attributed to differences in palmitic acid, which, as previously mentioned, is one of the major SFA responsible for increasing plasma low-density lipoprotein and total cholesterol concentrations in the human body. Whereas in the subcutaneous fat, where the concentration of palmitic acid was significantly lower in steers fed corn DDGS as opposed to steers fed wheat DDGS, the concentration of palmitic acid in the intramuscular fat was similar among steers fed a DDGS diet. Therefore, the lower levels of palmitic acid found in steers fed corn DDGS in the subcutaneous fat are negated in the intramuscular fat, leading to the differences between subcutaneous and intramuscular fat in terms of total SFA contents.

In contrast to the subcutaneous fat of steers fed various DDGS diets, dietary differences were observed for MUFA concentrations in the intramuscular fat **(Table 3-8)**. Steers fed the control diet displayed higher (P<0.001) levels of MUFA, which can be attributed to higher (P<0.001) levels of *cis*-MUFA in the intramuscular fat of steers fed the control diet as opposed to steers fed a DDGS diet. Although there are dietary differences in the total MUFA contents of the intramuscular fat, total levels of MUFA in the intramuscular fat decreased in comparison to the levels shown in the subcutaneous fat. This decrease in total MUFA can be attributed to a decrease in total *cis* MUFA concentrations, as well as to an increase in total PUFA concentrations, which is due to increased levels of PUFA present in the phospholipid portion combined with the inhibition of Δ^9-desaturase by PUFA (Aldai et al., 2010a; Griinari et al. 2000).

Table 3-8 Percent unsaturated fatty acid composition of intramuscular fat from steers fed various DDGS diets.

Fatty acid, [1] wt %	Treatment				SEM	*P*-value
		DDGS Diet				
	Control	Corn	Wheat	Blend		
∑ MUFA	45.94[a]	42.96[b]	42.04[b]	43.00[b]	0.637	<0.001
∑ *cis* MUFA	42.55[a]	37.26[b]	38.43[b]	39.23[b]	0.691	<0.001
∑ *trans* MUFA	3.39[b]	5.70[a]	3.61[b]	3.77[b]	0.228	<0.001
6*t*/7*t*/8*t*-18:1	0.175[c]	0.444[a]	0.245[bc]	0.268[b]	0.023	<0.001
9*t*-18:1	0.228[b]	0.434[a]	0.279[b]	0.288[b]	0.018	<0.001
10*t*-18:1	1.86[b]	2.84[a]	1.57[b]	1.55[b]	0.177	<0.001
11*t*-18:1	0.510[b]	1.06[a]	0.710[b]	0.864[b]	0.043	<0.001
12*t*-18:1	0.111[c]	0.229[a]	0.168[b]	0.182[b]	0.009	<0.001
13*t*/14*t*-18:1	0.263[c]	0.411[a]	0.359[ab]	0.352[b]	0.014	<0.001
15*t*-18:1	0.145	0.173	0.155	0.148	0.011	0.283
16*t*-18:1	0.097[b]	0.107[ab]	0.127[a]	0.123[ab]	0.008	0.029
11*t*/10*t*-18:1 ratio	0.327[b]	0.423[b]	0.504[ab]	0.627[a]	0.053	0.001
∑ n-3 PUFA	1.17[ab]	0.989[b]	1.24[a]	0.998[b]	0.055	0.003
18:3n-3	0.394[b]	0.357[b]	0.486[a]	0.396[b]	0.016	<0.001
22:5n-3	0.433	0.355	0.431	0.357	0.027	0.063
∑ n-6 PUFA	5.83[b]	10.30[a]	10.94[a]	10.31[a]	0.462	<0.001
18:2n-6	4.10[b]	8.30[a]	8.66[a]	8.22[a]	0.351	<0.001
18:3n-6	0.088	0.096	0.104	0.093	0.009	0.649
20:2n-6	0.106[b]	0.129[ab]	0.137[a]	0.138[a]	0.008	0.017
20:3n-6	0.314[b]	0.393[ab]	0.465[a]	0.392[ab]	0.024	<0.001
20:4n-6	1.07	1.21	1.38	1.29	0.09	0.095
22:4n-6	0.159	0.17	0.193	0.176	0.013	0.31
∑ PUFA	7.00[b]	11.29[a]	12.18[a]	11.31[a]	0.506	<0.001
n-6:n-3	5.02[c]	10.48[a]	9.01[b]	10.28[a]	0.269	<0.001

[1] Fatty acids <0.05% of total fatty acids not reported. MUFA: monounsaturated fatty acids: ∑ MUFA = *c*9-14:1 + *c*9-15:1 + *c*7-16:1 + *c*9-16:1 + *c*9-17:1 + *c*9-18:1 + *c*11-18:1 + *c*12-18:1 + *c*13-18:1 + *c*14-18:1 + *c*15-18:1 + *c*9-20:1 + *c*11-20:1 + *t*6/*t*7/*t*8-18:1 + *t*9-18:1 + *t*10-18:1 + *t*11-18:1 + *t*12-18:1 + *t*13-*t*14-18:1 + *t*15-18:1 + *t*16-18:1; ∑ cis MUFA = c9-14:1 + c9-15:1 + c7-16:1 + c9-16:1 + c9-17:1 + c9-18:1 + c11-18:1 + c12-18:1 + c13-18:1 + c14-18:1 + c15-18:1 + c9-20:1 + c11-20:1; ∑ trans MUFA = t6/t7/t8-18:1 + t9-18:1 + t10-18:1 + t11-18:1 + t12-18:1 + t13-t14-18:1 + t15-18:1 + t16-18:1. PUFA = polyunsaturated fatty acids: ∑ n-3 PUFA = 18:3n-3 + 20:3n-3 + 22:5n-3; ∑ n-6 PUFA = 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 + 20:4n-6 + 22:4n-6; ∑ PUFA = n-3 PUFA + n-6 PUFA; *c* = *cis*; *t* = *trans*.

Although concentrations of total and *cis* MUFA decreased from the subcutaneous to intramuscular fat, the total concentration of *trans* MUFA increased from the subcutaneous to intramuscular fat. Feeding corn DDGS to steers resulted in the highest (P<0.001) total *trans* MUFA content, as well as higher levels of individual *trans*-18:1 (6*t*/7*t*/8*t*-, 9*t*-, 10*t*-, 11*t*-, 12*t*-, 13*t*/14*t*-18:1) when compared to the intramuscular fat from steers fed other dietary treatments. However, this increase in total *trans* MUFA was accompanied by an increase in 11*t*-/10*t*-18:1 ratio. It was subsequently found that feeding steers a blend DDGS diet still resulted in a higher (P<0.05) (more desirable) ratio, while feeding steers a control diet resulted in the lowest (least desirable) ratio.

Accompanied by the increase in total *trans* MUFA, total concentrations of PUFA increased from the subcutaneous to the intramuscular fat. This, as previously mentioned, can account for decreases in total SFA and MUFA concentrations in the intramuscular fat of steers fed various DDGS diets. In this fashion, the intramuscular fat of steers fed a DDGS diet contained higher (P<0.001) concentrations of PUFA than the intramuscular fat of steers fed the barley control diet. This increase in total levels of PUFA in the intramuscular fat was also beneficial for the n-6:n-3 ratio, lowering the ratio for all diets when compared to the n-6:n-3 ratio in the back fat. As was observed in the subcutaneous fat, feeding steers a corn or blend DDGS diet resulted in the highest (P<0.001) ratio, while feeding steers a wheat DDGS diet resulted in an intermediate ratio, and feeding steers the control diet resulted in the lowest ratio.

Similar to the occurrence in PUFA, there was an increase in total CLA from the subcutaneous to the intramuscular fat of steers fed all of the diets except for corn DDGS **(Table 3-9)**. Even though there was an increase in total CLA for the control and wheat-based DDGS diets, the intramuscular fat of steers fed corn DDGS still exhibited higher (P<0.001) concentrations of CLA than steers fed blend DDGS and the control diet. In contrast to subcutaneous fat, the higher concentrations of CLA in the intramuscular fat of steers fed corn DDGS cannot be attributed to higher concentrations of one specific fatty acid, but rather to slightly higher concentrations in all of the major CLA isomers.

Table 3-9 Percent conjugated linoleic acid (CLA) composition of intramuscular fat from steers fed various DDGS diets.

Fatty acid, wt %	Treatment				SEM	*P*-value
		DDGS Diet				
	Control	Corn	Wheat	Blend		
∑ CLA	0.685[b]	0.876[a]	0.781[ab]	0.758[b]	0.030	<0.001
7*t*,9*c*-18:2	0.099[b]	0.175[a]	0.123[b]	0.117[b]	0.009	<0.001
9*c*,11*t*-18:2	0.372	0.457	0.429	0.434	0.022	0.054
9*t*,11*c*-18:2	0.103[a]	0.094[ab]	0.083[ab]	0.071[b]	0.007	0.011
10*t*,12*c*-18:2	0.022[b]	0.057[a]	0.046[a]	0.048[a]	0.004	<0.001
11*t*,13*c*-18:2	0.013	0.011	0.012	0.01	0.001	0.417
11*c*,13*t*-18:2	0.008	0.007	0.007	0.006	0.000	0.410
8*t*,10*c*-18:2	0.012	0.013	0.013	0.012	0.001	0.533

∑ CLA = t9,c11-18:2 + c9,t11-18:2 + t7,c9-18:2 + t10,c12-18:2 + t11,c13-18:2 + c11,t13-18:2 + t8,c10-18:2 + (c,t-t,c)12,14-18:2 + t12,t14-18:2 + t11,t13-18:2 + t10,t12-18:2 + t9,t11-18:2.

In summary, major differences were detected in fatty acid composition between the subcutaneous and intramuscular fat, which can be attributed to higher concentrations of PUFA in the intramuscular fat. Total SFA and MUFA concentrations were lower in the intramuscular fat than was observed in the subcutaneous fat; however, total *trans* MUFA and CLA concentrations were higher in the intramuscular fat than in the subcutaneous fat. Likewise, more desirable 11*t*-/10*t*-18:1 and n-6:n-3 ratios were observed in the intramuscular fat as opposed to the subcutaneous fat.

It appeared that the intramuscular fatty acid profile of steers fed blend DDGS closely resembled that of steers fed wheat DDGS, with total SFA concentrations being lower in the intramuscular fat of steers fed a DDGS diet containing wheat DDGS as opposed to the control diet, and with levels of 16:0 being lower in the intramuscular fat of steers fed a DDGS diet as opposed to steers fed the control diet. Likewise, the intramuscular fat of steers fed the barley control diet exhibited higher concentrations of MUFA than steers fed a DDGS diet, although the intramuscular fat of steers fed corn DDGS had higher concentrations of *trans*-MUFA than the intramuscular fat from steers fed the other diets. Feeding steers a diet containing DDGS fermented from wheat also resulted in a more desirable 11*t*-/10*t*-18:1 ratio, while feeding steers a DDGS diet resulted in higher concentrations of PUFA in the intramuscular fat than feeding steers the barley control diet. Lastly, total concentrations of CLA in the intramuscular fat did not differ

between steers fed corn and wheat DDGS, while total concentrations did not differ between steers fed wheat and blend DDGS; therefore, it can be concluded that substituting 40% wheat DDGS or a blend of wheat and corn DDGS for barley grain in the finishing diet of steers will improve the fatty acid profile of the resulting intramuscular fat.

3.4.3 Vitamin E

According to Faustman et al. (1998), vitamin E is the collective generic name for "all entities that exhibit the biological activity of α-tocopherol." Alpha-tocopherol is a membrane-associated antioxidant that protects the vulnerable unsaturated fatty acids in cell membranes and plasma lipoproteins from endogenous and exogenous oxidizing agents (Guo et al., 2006). As lipids oxidize, free radicals are produced, advancing the oxidation of oxymyoglobin and deoxymyoglobin, which leads to the development of rancid off-flavours and odours, and a brownish discolouration during retail display (Yang et al., 2002).

The most effective method of reducing lipid oxidation in precooked meats is accomplished by supplementing diets with vitamin E because the vitamin is incorporated into the membrane structures, thereby protecting the phospholipids against oxidative attack (Jensen et al., 1998). Previous studies (Faustman et al., 1998; Liu et al., 1996) have also shown that dietary supplementation of vitamin E increases muscle α-tocopherol concentrations, thus extending the shelf-life by prolonging colour stability. Other researchers (Gray et al., 1996) reported that high levels of α-tocopherol acetate in ground beef delays the development of oxidation by 1.6 to 5 days.

In the present study, higher ($P<0.05$) levels of total vitamin E were detected in the intramuscular fat of steers fed corn DDGS as opposed to steers fed wheat DDGS and the barley control diets **(Table 3-10)**. This difference can be attributed to the presence of γ-tocopherol in the intramuscular fat of steers fed corn DDGS as opposed to the control and wheat DDGS diets, where γ-tocopherol was not detected. Likewise, the blend DDGS diet produced an intermediate result due to the presence of corn during fermentation, which allowed for the incorporation of a small amount of γ-tocopherol into the DDGS, which was incorporated into the intramuscular fat of steers. When comparing α-tocopherol

concentrations in the fat of ground beef obtained from steers fed DDGS versus WDGS at 20 or 40% of the dietary dry matter, Koger et al. (2010) did not find any detectable differences in steers fed DDGS compared with steers fed WDGS, despite finding higher levels of α-tocopherol in the DDGS diet as opposed to the WDGS diet.

Table 3-10 Vitamin E concentrations of intramuscular fat from steers (n=80) fed various DDGS diets.

	Treatment				SEM*	*P*-value
		DDGS Diet				
	Control	Corn	Wheat	Blend		
∑ Vitamin E (μg/g)	1.87[b]	2.54[a]	1.98[b]	2.15[ab]	0.138	0.006
α-Tocopherol	1.68	2.02	1.94	1.90	0.105	0.128
α-Tocotrienol	0.424	0.455	0.390	0.342	0.043	0.090
γ-Tocopherol	0.000[c]	0.403[a]	0.000[c]	0.165[b]	0.022	<0.001

*Pooled SEM

3.4.4 Lipid Oxidation

The development of rancid off-flavours and odours during retail display is typically attributed to lipid oxidation (Lynch et al., 2000). Oxidation of the lipid components affects fatty acids, particularly PUFA (Gill et al., 2008), due to a higher degree of unsaturation. One lipid oxidation product that has received the greatest attention is malondialdehyde because it can be detected by the 2-thiobarbituric acid reactive substances (TBARS) test (Koger et al., 2010).

Results for TBARS analysis on raw and cooked ground beef are presented in **Table 3-11**. From this analysis, it is evident that diet had no effect on the oxidation of ground beef obtained from the *longissimus* muscle of steers fed various DDGS diets. Findings from Depenbusch et al. (2009) support these findings because they reported that TBARS values did not differ in the *longissimus* muscle of heifers fed increasing levels of DDGS (up to 75%). In contrast, Gill et al. (2008) found that feeding yearling steers corn DDGS resulted in greater amounts of lipid oxidation in the striploin when compared with steers fed corn WDGS; this result is due to the meat obtained from steers being fed corn DDGS having greater concentrations of PUFA. Similarily, Koger et al. (2010) found that ground beef from steers fed 40% DDGS had increased TBARS when compared to ground beef of steers fed a control diet.

Table 3-11 TBARS values of raw and cooked ground beef obtained from steers (n=48) fed various DDGS diets.

	Treatment				SEM	Day			SEM*	*P*-value		
		DDGS Diet										
	Control	Corn	Wheat	Blend		1	3	7		Diet	Day	Diet x Day
TBARS [1]												
Raw [2]	5.14	4.84	4.48	5.36	0.459	2.58	5.03	7.25	0.281	0.504	<0.001	0.538
Cooked	4.72	4.32	4.35	4.46	0.122	2.60	4.68	6.11	0.066	0.104	<0.001	0.068

* Pooled SEM

[1] mg malondialdehyde per kilogram of beef

[2] Used data from a subset of steers slaughtered on weeks 2, 3 and 4 (n=36).

The reason why a dietary effect was not evident in either the cooked or raw ground beef is not certain, despite the fact that the meat obtained from steers fed a DDGS had almost twice as much PUFA as steers fed the barley control diet. One possible explanation is that feeding DDGS allowed for the elevated incorporation of vitamin E into the phospholipid membranes; however, the results from α-tocopherol analysis do not support this theory due to the fact that meat from steers fed corn and blend DDGS had higher levels of vitamin E than meat from steers fed the control or wheat DDGS, yet no differences in TBARS was observed.

When looking at the raw TBARS values, in particular, there is a large difference between the highest and lowest mean values. These differences would typically be large enough for significance to be detected. This statement is supported by research from Koger et al. (2010), who found significant dietary differences in ground beef separated by less than one TBARS unit. However, there is a lot of variation in the data, as indicated by the large SEM value, so animal variation might have been too large to detect any statistical dietary differences. Increased animal variation can be attributed to a reduced sample size (i.e., data from nine rather than twelve per treatment group) which, in turn, reduces the power of the experiment. By increasing the sample size, variation within the data is reduced and the power of the experiment of the experiment is increased; however, difficulties encountered during analysis led to the reduced sample numbers for analysis.

Although no dietary differences were detected, a day difference was detected for both raw and cooked ground beef. This was expected because meat stored under aerobic

conditions allows oxygen to react with the unsaturated fatty acids to produce free radicals, thus increasing the levels of lipid oxidation products. What was not expected were the higher TBARS values for raw ground beef as opposed to cooked ground beef. Typically, cooking expediates the onset of lipid oxidation, resulting in higher TBARS values; however, in this study the raw meat exhibited higher TBARS values than the cooked meat. These higher TBARS values can possibly be attributed to sample preparation.

Following grinding, the raw meat designated for cooked TBARS analysis was vacuum-packaged and frozen. John et al. (2005) have shown that vacuum-packaging results in decreased lipid oxidation for cooked sirloin steaks during refrigerated storage, while fat oxidation proceeds at a slower rate in frozen meats as opposed to refrigerated meats (Tan and Shelef, 2002); therefore, the combination of vacuum-packaging and freezing may have been adequate to stop the development of lipid oxidation products. Then, prior to exposure to oxygen, the ground beef was cooked in a water bath. It is hypothesized that cooking under anaerobic conditions may have allowed for the development of other antioxidant compounds, such as Maillard reaction products, so by the time the cooked ground beef was exposed to oxygen, it may have had adequate antioxidant compounds to limit the development of lipid oxidation products.

3.4.5 Retail Display

3.4.5.1 Objective Evaluation

Visual appearance is one of the critical properties influencing a consumer's purchasing decision (Lui et al., 1996), thus the ability of meat to stabilize and maintain colour is the most important quality attribute of retail shelf life (Koger et al., 2010). The colour coordinate values of L* (lightness), a* (redness) and b* (yellowness) for the present study are presented in **Table 3-12**. Feeding regime had no effect on b*; however, there was a diet by day effect for both L* and a* ($P<0.05$), with the colour coordinate values for L* and a* gradually decreasing ($P<0.05$ and <0.001, respectively). This gradual decrease in colour measurements can be attributed to meat colour deterioration as a result of lipid oxidation and a loss of reducing ability (Gill et al., 2008).

Table 3-11 Effect of diet on objective retail measurements of *longissimus* steaks[1] obtained from steers fed various DDGS diets when stored under retail display conditions (4°C; 7 d).

	Treatment				SEM[2]	Day								SEM[2]	*P*-value		
	DDGS Diet																
	Control	Corn	Wheat	Blend		0	1	2	3	4	5	6	7		Diet	Day	Diet x Day
L*[3]	40.89[ab]	41.03[ab]	42.05[a]	39.37[b]	0.665	42.70	41.24	40.85	40.36	40.51	40.40	40.38	40.20	0.354	0.046	<0.001	0.031
a*[4]	27.17[a]	24.60[b]	25.08[b]	23.80[b]	0.546	32.02	31.07	29.76	27.66	25.79	22.73	17.98	14.06	0.323	<0.001	<0.001	0.002
b*[5]	22.22	21.44	21.48	21.01	0.324	24.86	24.91	23.76	22.00	21.21	19.94	18.32	17.22	0.203	0.056	<0.001	0.187

[1] 20 steaks / treatment; 19 steaks for control because dark cutter was removed from analysis.

[2] Pooled SEM

[3] L* lightness (0 = black, 100 = white)

[4] a* redness (positive values = red, negative values = green)

[5] b* yellowness (positive values = yellow, negative values = blue)

Overall, retail steaks obtained from steers fed wheat DDGS were lighter (higher L* value; $P<0.05$) than steaks obtained from steers fed blend DDGS, while steaks obtained from steers fed control and corn DDGS displayed intermediate values. Early research conducted by Boles et al. (2004) found that steers fed corn-based diets had steaks that were darker (lower L* values) than steaks obtained from steers fed diets formulated with various barley varieties. Other research conducted by Gill et al. (2008) found that steaks obtained from steers fed a steam flaked corn diet were darker across retail display than steaks obtained from steers fed a diet containing distillers grains. Roeber et al. (2005) also observed that steaks obtained from steers fed diets supplemented with 20% DDG, 40% DDG and 40% WDG were lighter than steaks obtained from steers fed 10% of either DDG or WDG. In contrast, Aldai et al. (2010b) did not find any dietary differences in the colour of steaks obtained from steers fed corn or wheat DDGS; however, they did observe that retail steaks obtained from animals fed 40% corn DDGS had a lighter colour than steaks obtained from animals fed 20% corn DDGS.

Steaks obtained from steers fed a DDGS diet were not as red (lower a* value; $P<0.05$) as steaks obtained from steers fed the barley control diet. These findings are in agreement with Roeber et al. (2005), who found that the red colour (a* values) of steaks was enhanced when Holstein steers were fed diets containing corn DDGS at 10 or 25% of the dietary DM compared with steaks obtained from steers fed corn grain-based diets; however, the red colour was negatively affected when corn DDGS was included at 40% of the dietary dry matter. Gill et al. (2008) obtained similar results when they found that striploin steaks obtained from steers fed corn or sorghum DDGS or WDGS at 15% dietary inclusion were not as red as steaks obtained from steers fed steam-flaked corn. In contrast, Depenbusch et al. (2009) found that redness values were not different for steaks obtained from cattle fed different levels of DDGS until 7 d retail display, when the redness of steaks linearly decreased as a result of feeding higher levels of DDGS. Koger et al. (2010) and Kinman et al. (2011) did not find any differences in a* values of steaks obtained from steers fed DDGS when stored under retail conditions.

When evaluating b*, there was a trend ($P<0.1$) towards having an effect on yellowness; however, the dietary differences were not significant. These findings are in

accordance with Koger et al. (2010), who did not observe any dietary differences in b* values in steaks obtained from heifers fed increasing levels of DDGS. In contrast, Gill et al. (2008) found that steaks obtained from steers fed steam-flaked corn were yellower (higher b* values) across the period of retail display than steaks obtained from steers fed diets containing DDGS. More specifically, Gill et al. (2008) found that sorghum WDDS diets produced steaks with greater b* values than steaks obtained from steers fed sorghum DDGS. Likewise, Roeber et al. (2008) found steaks obtained from steers fed corn WDGS at 12.5% or 25% of the diet DM were yellower than steaks obtained from steers fed the control of 25% corn DDGS.

A diet by day interaction was also observed for the L* colour parameter **(Figure 3-1)**. When evaluating the effect of feeding increasing levels of DDGS on the colour stability of the *longissimus* muscle obtained from yearling heifers, Depenbusch et al. (2009) observed that steaks became darker at DDGS levels higher than 45%. However, in the current study, the effect of DDGS on lightness was dependent on the type of DDGS that was fed to the cattle. Day 0 readings agree with the dietary findings, where steaks obtained from steers fed wheat DDGS were significantly lighter than steaks obtained from steers fed blend DDGS. The day 0 L* readings were also significantly lighter than the day 1 L* readings for steaks obtained from steers fed the control, corn DDGS and wheat DDGS diets. Aside from the initial decrease in lightness, the L* value decreased at a similar rate for all of the steaks, regardless of diet.

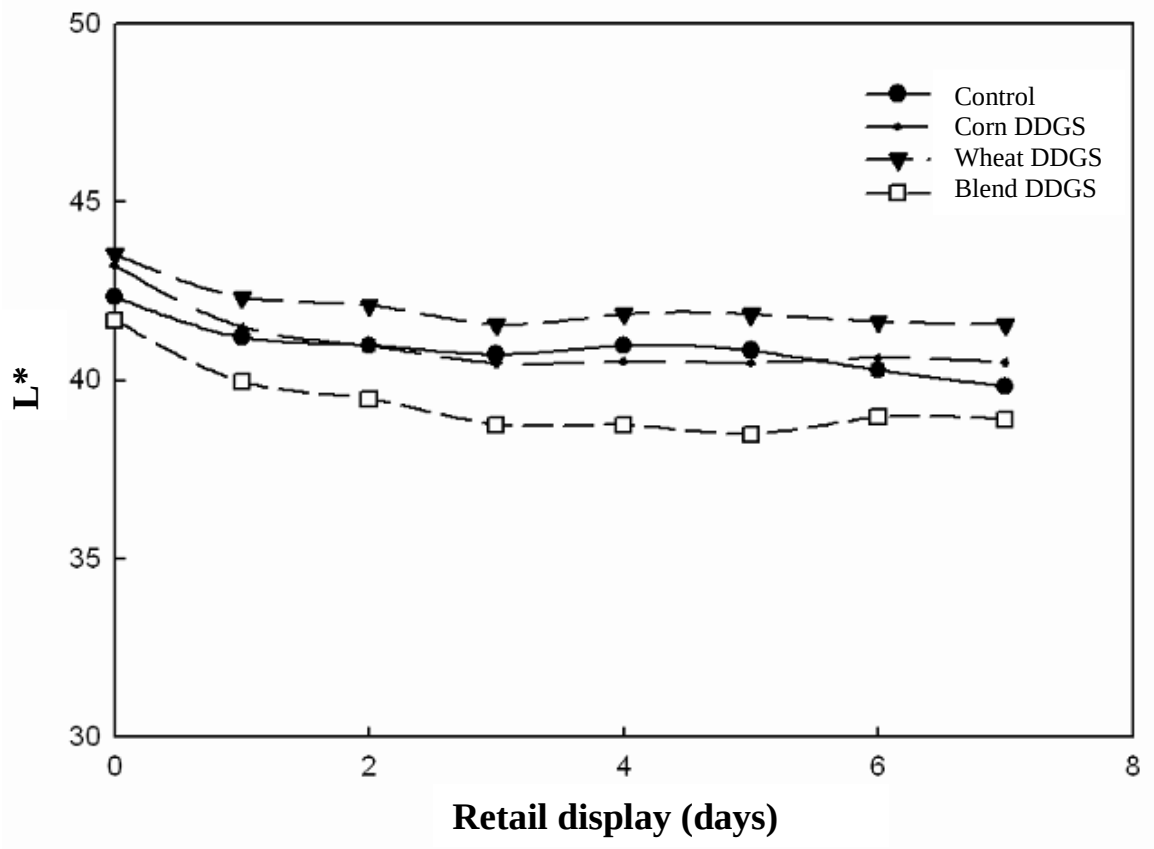

Figure 3-1 Effects of feeding the control or wheat, corn or blend dried distillers grains plus solubles (DDGS) diets to crossbred beef steers on L* after 7 days of simulated retail display (higher values = more white) values of *longissimus* steaks (n = 79). One steak (control) was removed from analysis as it was identified as a dark cutter.

Likewise, a diet by day interaction was observed for the a* colour parameter **(Figure 3-2)**. There were no differences in regards to redness until day 4, when the steaks obtained from steers fed DDGS were not as red as steaks obtained from steers fed the barley control diet. This trend was observed throughout the remainder of retail display, with steaks obtained from steers fed the barley control diet retaining their colour better than the steaks obtained from steers fed a DDGS diet. However, there was some differentiation in the colour deterioration of steaks obtained from steers fed a DDGS diet. Specifically, steaks obtained from animals fed a DDGS diet fermented from corn lost redness at a faster rate than steaks obtained from animals fed wheat DDGS. Although steaks obtained from steers fed a DDGS diet were not as colour stable as steaks obtained from steers fed a barley diet, feeding wheat DDGS leads to enhanced meat colour stability over feeding corn DDGS or a DDGS diet fermented from both wheat and corn.

A diet by day effect was also observed by Leupp et al. (2009), where the a* values for steaks obtained from steers fed 30% DDGS decreased at a faster rate than the a* values for steaks obtained from steers fed 0% DDGS. According to Gray et al. (1996), the reason for a faster decline in a* of steaks from steers fed DDGS may be attributed to increased oxidation of the unsaturated fatty acids and enzymatic reducing systems that control metmyoglobin levels in meat.

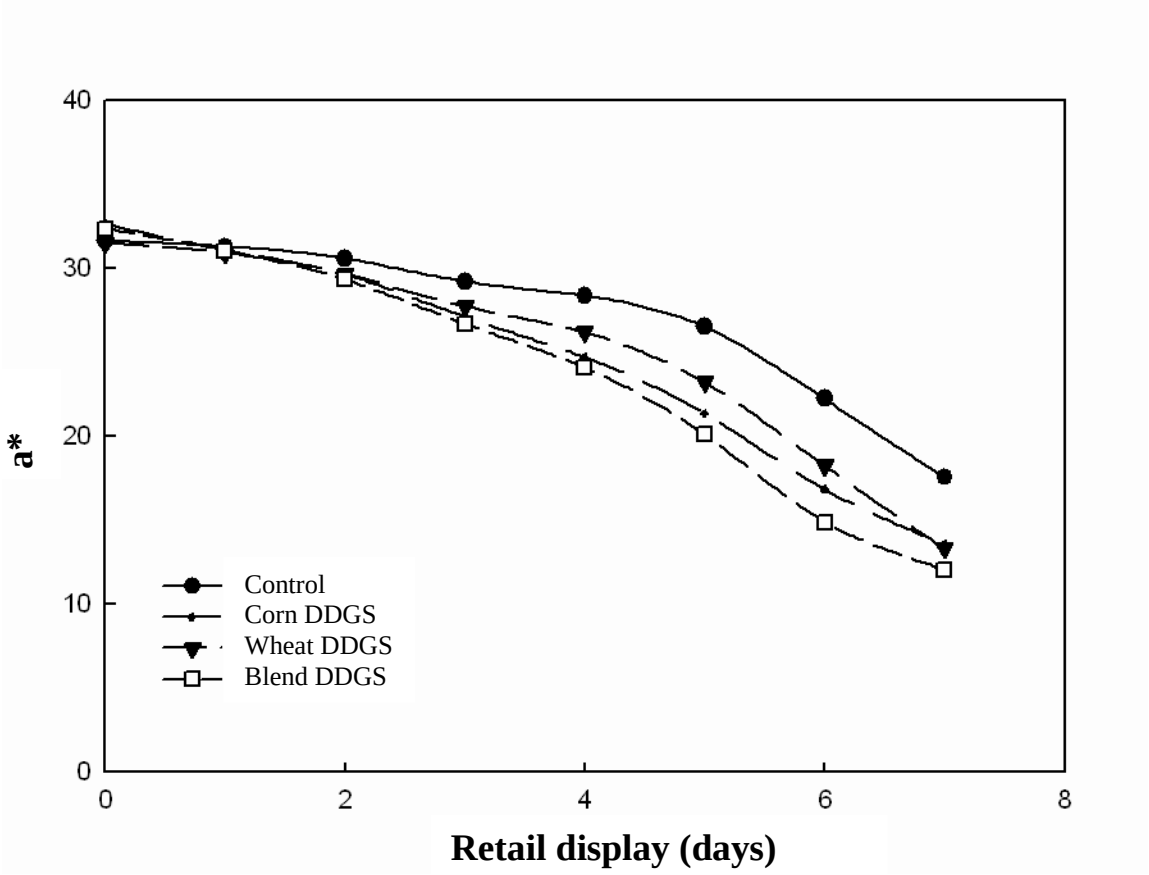

Figure 3-2 Effects of feeding the control or wheat, corn or blend dried distillers grains plus solubles (DDGS) diets to crossbred beef steers on a* (higher values = more red, lower values = more green) values of *longissimus* steaks after 7 days of simulated retail display (n = 79). One steak (control) was removed from analysis as it was identified as a dark cutter.

Effect of days of retail display was also significant (P<0.001) for all of the objective measurements recorded. Under retail conditions, all steaks showed a gradual decrease in all measured colour parameters, which is in accordance with data obtained by Aldai et al. (2010b), who observed a shift towards lower L*, lower chroma (colour intensity) and a more brownish hue.

3.4.5.2 Subjective Evaluation

As previously documented by Zerby et al. (1999), objective colour measurements are in good accordance with visual appearance, or subjective colour measurements. Gill et al. (2008) did not observe any treatment or treatment by time differences in visual colour scores during simulated retail display of steaks obtained from beef cattle fed 15% (DM basis) corn or sorghum distillers grains; however, they did observe that the visual colour scores for all dietary treatments decreased during retail display due to lipid oxidation that induced meat colour deterioration. Similar results were obtained by Aldai et al. (2010b), who found that, over time, all retail steaks obtained from steers fed 20% or 40% corn or wheat DDGS showed a shift towards a less desirable retail appearance due to a darker colour and higher percentage of surface discolouration.

In the present study, a dietary effect was not observed for percent surface discolouration, discolouration colour, amount of marbling, or marbling colour; however, a dietary difference was detected for overall retail appearance and lean colour score **(Table 3-13)**. More specifically, steaks obtained from steers fed the barley control diet were scored as having a more desirable retail appearance than steaks obtained from steers fed blend DDGS, while steaks obtained from steers fed wheat DDGS were scored as having a visually lighter lean colour than steaks obtained from steers fed blend DDGS. These differences are supported by colour differences detected in the objective colour analysis.

Table 3-13 Effect of diet on subjective retail measurements of *longissimus* steaks[1] obtained from steers fed various DDGS diets when stored under retail display conditions (4°C; 7 d).

Descriptor*	Treatment				SEM[2]	Day					SEM[2]	*P*-value		
	DDGS Diet													
	Control	Corn	Wheat	Blend		1	2	3	4	7		Diet	Day	Diet x Day
Overall retail appearance	5.02[a]	4.61[ab]	4.55[ab]	4.41[b]	0.138	5.93	5.56	5.16	4.68	1.88	0.089	0.018	<0.001	0.028
Lean colour score	6.01[ab]	6.11[ab]	5.7[b]	6.36[a]	0.151	5.74	5.82	5.88	6.05	6.74	0.088	0.027	<0.001	0.240
Surface discolouration (%)	2.03	2.30	2.23	2.46	0.143	1.22	1.23	1.57	2.06	5.22	0.092	0.205	<0.001	0.008
Discolouration colour	2.34	2.31	2.27	2.45	0.171	1.43	1.50	1.97	2.74	4.08	0.111	0.887	<0.001	0.622
Amount of marbling	2.73	2.79	2.73	2.71	0.123	2.60	2.69	2.70	2.76	2.96	0.068	0.967	<0.001	0.629
Marbling colour	2.43	2.46	2.40	2.53	0.081	2.59	2.54	2.76	2.59	1.81	0.062	0.682	<0.001	0.093

* Overall retail appearance (1 = extremely undesirable and 8 = extremely desirable); Lean colour score (1 = white and 8 = extremely dark red); Percent surface discolouration (1 = 0% and 7 = 100% discolouration); Discolouration colour (1 = no browning and 7 = black); Amount of marbling (1 = devoid and 6 = abundant); Marbling colour (1 = white and 5 = brown).

[1] 20 steaks / treatment; 19 steaks for control because dark cutter was removed from analysis.

[2] Pooled SEM

Because visual appearance is the most critical property influencing a consumer's purchasing decision, it can be expected that steaks obtained from steers fed the barley control diet would be scored as having the most desirable overall retail appearance because they were the steaks best able to retain their colour (redness) over retail display. In contrast, steaks obtained from steers fed blend DDGS displayed the lowest overall a* values; therefore, these steaks underwent colour deterioration at a more rapid rate, leading to the least acceptable retail appearance.

Similarily, steaks obtained from steers fed wheat DDGS were lighter (higher L* value) than steaks obtained from steers fed blend DDGS. Therefore, the lean colour score differences, as detected by trained panelists, only serve to reinforce the findings via objective colour analysis.

Diet by day interactions were also detected for overall retail appearance and percent surface discolouration (**Figure 3-3** and **3-4**, respectively). In this study, an overall retail appearance score of 3 (moderately unacceptable) is when the steaks were assumed to be discounted for quick sale in retail display (Roeber et al., 2005). In a study conducted by Roeber et al. (2005), they found that it took approximately 5.5 d of simulated retail display before strip loin steaks obtained from Holstein steers were scored as moderately unacceptable. As can be seen on the graph, feeding the barley control diet to steers helped to maintain the overall retail appearance when compared to feeding blend DDGS. The increased colour deterioration of steaks obtained from steers fed blend DDGS can be attributed to the inability to retain colour (lower a* value) as well as to increased surface discolouration. It is interesting to note that feeding corn or wheat DDGS did not negatively affect the overall retail appearance of steaks, as the values did not significantly differ from steers fed the control diet.

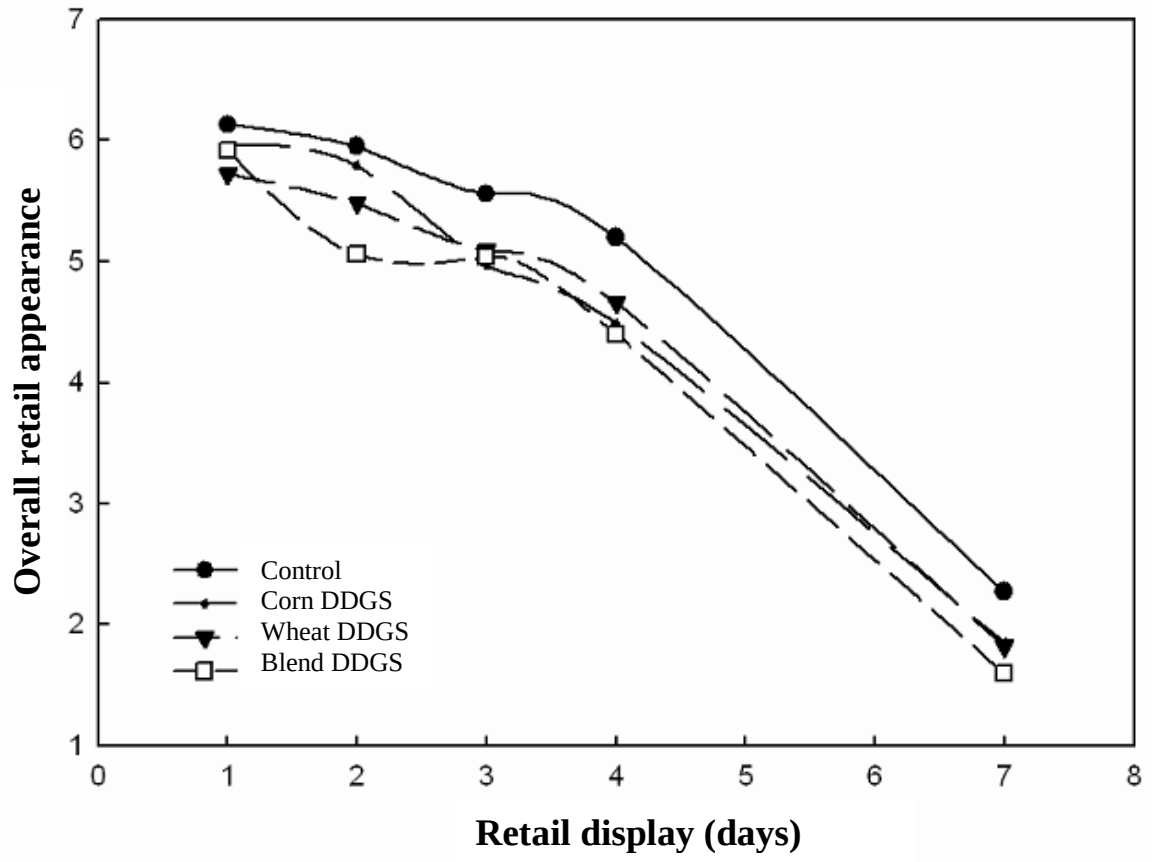

Figure 3-3 Effects of feeding the control or wheat, corn or blend dried distillers grains plus solubles (DDGS) diets to crossbred beef steers on overall retail appearance (8 = extremely desirable, 1 = extremely undesirable) values of *longissimus* steaks after 7 days of simulated retail display (n = 79). One steak (control) was removed from analysis as it was identified as a dark cutter.

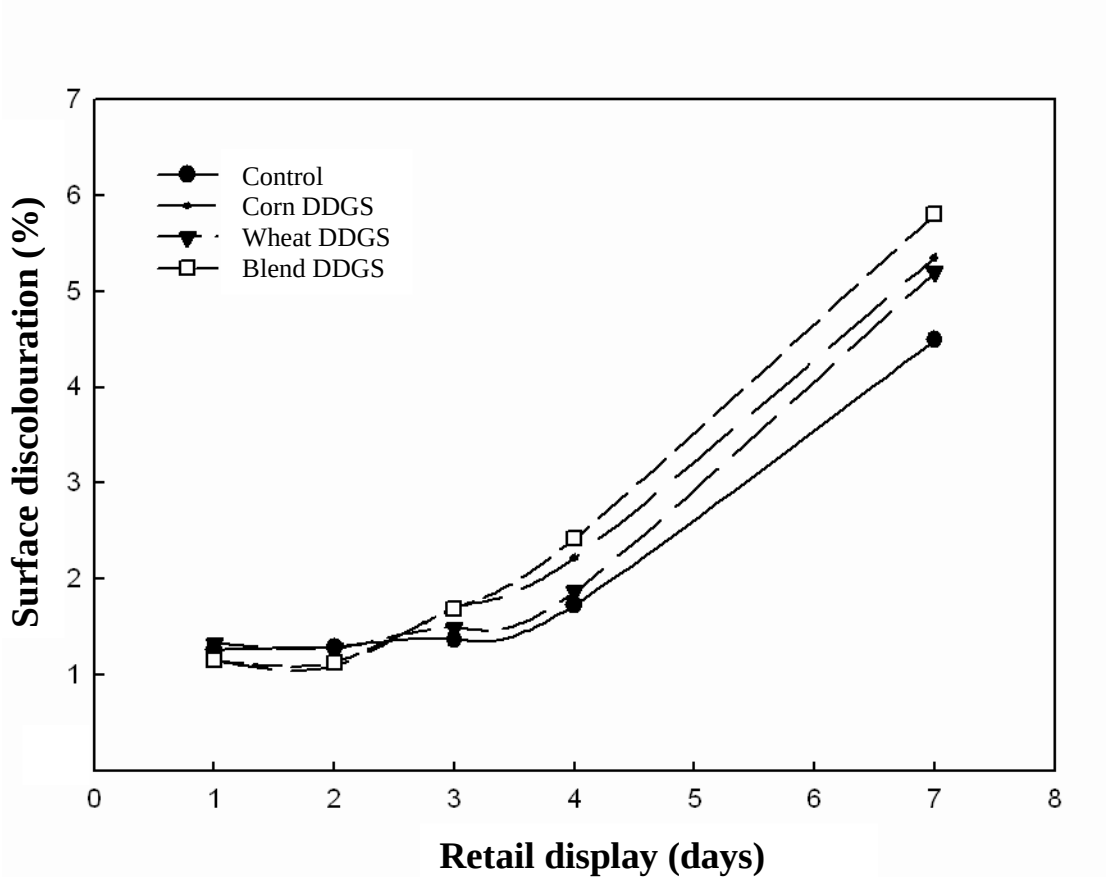

Figure 3-4 Effects of feeding the control or wheat, corn or blend dried distillers grains plus solubles (DDGS) diets to crossbred beef steers on percent surface discolouration (1 = 0%, 7 = 100%) values of *longissimus* steaks after 7 days of simulated retail display (n = 79). One steak (control) was removed from analysis as it was identified as a dark cutter.

3.4.6 Sensory Analysis

In this study, no dietary differences were detected for any of the sensory parameters, including any of the flavour or texture descriptor percentages **(Table 3-14)**. This could be due to the steaks in the current experiment being subjected to a year of frozen storage prior to analysis, mitigating any dietary differences. These findings are in accordance with Shand et al. (1998), who found that steaks from steers fed wheat-based distillers grains were no different in flavour, off-flavour intensity or amount of connective tissue when compared with steaks from steers fed wet brewers grains or barley. Similarily, Kinman et al. (2011) failed to observe any dietary differences in sensory parameters in steaks obtained from steers fed various amounts of wet and dry distillers grains. Leupp et al. (2009) also failed to find any differences in tenderness when

evaluating steaks obtained from steers fed a control versus 30% corn DDGS; however, they did observe that steaks obtained from steers fed 30% corn DDGS during the finishing period were juicier and more flavourful than steaks obtained from control steers.

Table 3-14 Effect of diet (treatment) on the sensory parameters of *longissimus* steaks (n=80) obtained from steers fed various DDGS diets.

Descriptor*	Treatment				SEM	*P*-value
		DDGS Diet				
	Control	Corn	Wheat	Blend		
Initial Tenderness	5.77	5.47	5.82	6.09	0.213	0.235
Initial Juiciness	5.62	5.28	5.50	5.55	0.154	0.454
Beef Flavour Intensity	5.22	5.10	5.20	5.29	0.098	0.601
Off-Flavour Intensity	8.13	8.18	8.21	8.18	0.095	0.955
Amount of Connective Tissue	8.01	8.16	8.18	8.08	0.067	0.281
Overall Tenderness	5.55	5.48	5.52	5.50	0.218	0.402
Sustainable Juiciness	5.61	5.35	5.54	5.55	0.114	0.387
Flavour Desirability	5.47	5.45	5.52	5.57	0.105	0.844
Overall Palatability	5.04	4.89	5.12	5.19	0.154	0.542
Flavour Descriptor Percentages						
Metallic	0.00	0.00	0.72	0.00	0.357	0.398
Off-Sour	16.85	17.26	12.05	20.21	3.32	0.383
Livery	1.34	1.43	1.46	0.71	0.917	0.932
Grainy	1.67	0.00	0.00	0.00	0.574	0.106
Bloody	3.33	3.84	2.08	0.00	1.23	0.130
Other	0.83	0.00	0.00	1.46	0.657	0.328
Unidentified	22.32	29.20	29.64	25.60	3.44	0.401
None	53.66	48.27	54.05	52.02	4.34	0.776
Texture Descriptor Percentages						
Typical Beef	85.65	90.00	89.43	80.18	4.09	0.307
Mushy	6.34	2.98	3.84	7.65	2.65	0.573
Mealy	0.00	1.43	1.55	1.67	0.925	0.549
Rubbery	5.71	1.67	1.55	3.57	2.82	0.695
Crumbly	2.29	3.93	3.63	6.94	1.64	0.243

* Initial and Overall Tenderness (1 = extremely tough and 8 = extremely tender); Initial and Sustainable Juiciness (1 = extremely dry and 8 = extremely juicy); Beef Flavour Intensity (1 = extremely bland and 8 = extremely intense); Off-Flavour Intensity (1 = extremely intense off-flavour and 9 = no off-flavour); Amount of Connective Tissue (1 = abundant and 9 = none detected); Flavour Desirability and Overall Palatability (1 = extremely undesirable and 8 = extremely desirable).

Other research conducted by Aldai et al. (2010b) contrasts the findings of the current study. More specifically, Aldai et al. (2010b) found that initial and overall tenderness scores were higher in meat obtained from animals fed corn DDGS when compared to meat obtained from animals fed the barley-based control diet; corn DDGS steaks were also perceived as having a lower amount of connective tissue and higher overall palatability ratings when compared to control samples. In their study, improved tenderness ratings of steaks derived from corn DDGS fed steers were related to the thicker back fat and slower rate of cooling that may have accelerated or maintained postmortem muscle metabolic activity, resulting in increased tenderization (Aldai et al., 2010b). Aldai et al. (2010b) also found that, in general, DDGS steaks were rated better for flavour desirability when compared to control steaks. However, other studies including distillers grains in a barley-based finishing diet have shown no dietary effects on the eating quality of the resulting meat (Gill et al., 2008; Roeber et al., 2005). These differences are likely due to changes in live animal growth, such as rate of gain, fat deposition and maturing patterns, which are dependent on the energy content of the diet (Aldai et al., 2010b). Because the consistency of DDGS is extremely variable, variations in the results can occur.

3.5 Summary

In summary, no dietary differences were observed for meat quality, sensory attributes, or oxidative stability. Diet did have an effect on colour attributes, with steaks from the wheat DDGS group being lighter (higher L* value) than steaks from blend DDGS, while control and corn DDGS steaks displayed similar, intermediate L* values. Likewise, the retail display panel indicated that wheat DDGS steaks had a lighter lean colour score than steaks from the other diets. It was also observed that steaks from animals fed a DDGS diet lost redness faster over time (lower a* values) and had a less desirable retail appearance than control steaks; however, blend DDGS and corn DDGS steaks lost redness more rapidly than those from animals fed wheat DDGS.

Fatty acid analysis of the intramuscular fat showed that there was no difference in the total fatty acids (mg fatty acids/g meat); however, there were some marked

differences in fatty acid composition when compared to the subcutaneous fat. The intramuscular fat from steers fed wheat DDGS showed higher levels of branched chain fatty acids (BCFA) than did the intramuscular fat from steers fed the other diets, while the intramuscular fat from steers fed wheat and blend DDGS displayed lower levels of SFA than the intramuscular fat from steers fed the control diet. In contrast to the subcutaneous fat, only steers fed corn DDGS exhibited higher levels of CLA than the control diet but the intramuscular fat from the cattle fed any DDGS diet did exhibit higher levels of PUFA than did animals fed the control diet. Steers fed corn DDGS also yielded higher levels of *trans* MUFA when compared to the other diets, including higher levels of 10*t*:18:1.

Overall, feeding 40% wheat DDGS, 40% corn DDGS or their blend did not significantly affect meat quality; however, wheat DDGS did offer enhanced colour stability and a more desirable fatty acid profile over corn DDGS. In contrast, feeding the DDGS diet fermented from wheat and corn resulted in the poorest colour stability but it did produce a desirable fatty acid profile closely resembling that of wheat DDGS.

3.6 Connection to the Next Study

A second study was carried out to examine the effect of DDGS diet on further processed meat products. Numerous research studies have been conducted regarding the effect of DDGS diet on *longissimus* quality; however, no work has been done to examine the effect of DDGS diet on other muscles, such as the *semimembranosus.* Therefore, a second study was undertaken to determine if diet has an effect on the meat quality parameters of raw or pre-cooked *semimembranosus* roasts and to determine if employing further oxidative challenges has an impact on the oxidative stability of the resulting meat.

4.0 A COMPARISON OF DISTILLERS GRAINS PLUS SOLUBLES DERIVED FROM WHEAT, CORN OR A WHEAT/CORN BLEND ON THE QUALITY OF RAW AND PRE-COOKED *SEMIMEMBRANOSUS* ROASTS

4.1 Abstract

The objective of this study was to compare meat quality traits of raw and pre-cooked beef *semimembranosus* (SM) roasts from crossbred beef steers fed diets containing barley (control) or 40% wheat, corn or blend dried distillers grains with solubles (DDGS). Left inside rounds from 48 steers (12 per diet) were frozen for one year before testing. A portion of each SM was injected with a salt/phosphate brine (20% extension to deliver 0.85% sodium chloride and 0.40% sodium tripolyphosphate). Injected and non-injected roasts were cooked in a water bath to 72°C. Proximate, pH, colour, Warner-Bratzler (WB) shear, water holding and thiobarbituric acid reactive substances (TBARS) analyses were conducted over 56 d of storage (4°C).

No dietary effects ($P<0.05$) were observed for meat quality, processing attributes, or WB shear values of the non-injected SM roasts. Roasts from animals fed the corn DDGS had the lowest brine pickup and the highest WB shear values.

Raw non-injected meat from steers fed a DDGS diet lost redness more rapidly than meat obtained from steers fed the control diet and was less oxidatively stable, as determined using TBARS. Oxidative changes due to diet were mitigated in the cooked injected roasts due to the chelating effect of phosphate, which also lowered overall TBARS values. TBARS values of the pre-cooked SM roasts remained below levels where rancidity is normally detected over 56 days of refrigerated storage at 4°C. Overall, feeding 40% wheat DDGS, 40% corn DDGS or 40% wheat/corn blend DDGS had no negative effects on meat quality of raw SM or value–added products such as pre-cooked enhanced roasts.

4.2 Introduction

Beef roasts are a popular food service item. The *semimembranosus* and *adductor* muscle, with or without cap (*gracilus* muscle), are being used for the manufacturing of beef roasts because they are large muscles with a consistent grain and have less marbling, which can interfere with cooked product appearance (Boles and Swan, 2002a). Currently, the market for convenience products is expanding; consequently, with the increasing popularity of these value-added products, there is a growing demand for chuck and round cuts, which are traditionally marketed as low priced steaks or roasts (Pietrasik and Shand, 2004). However, since these cuts have been described as the most under-valued wholesale cuts from the beef carcass (National Cattlemen's Beef Association, 2002), treatments to improve the tenderness of round muscles would add value to the whole carcass (Pietrasik and Shand, 2004). Therefore, the injection of various salt and phosphate formulations into primal meat cuts is routinely practiced to enhance the tenderness and juiciness of fresh meat products (Dhanda et al., 2002).

Numerous studies have been published regarding the effect of animal diet on the eating quality of beef (Bidner et al., 1986; Nour et al., 1994; Shand et al., 1998; Daly et al., 1999; Sapp et al., 1999; Gill et al., 2008; Leupp et al., 2009; Aldai et al., 2010b); however, most of these studies concentrate on the effect of diet on the eating qualities of fresh beef and neglect such effects on beef for further processing (Farouk and Wieliczko, 2003). In particular, no work has been done examining the effects of feeding ethanol by-products, such as dried distillers grains plus solubles (DDGS), on beef for further processing. This is especially important in cattle fed a DDGS diet because it has been documented that feeding a DDGS diet increases polyunsaturated fatty acid levels in the *longissimus* muscle (Chapter 3; Aldai et al., 2010a). Therefore, it is important to determine if this increase in PUFA will have an effect on further processed meats.

In Canada and the United States, feed grains are being increasingly used for ethanol production, which has increased feed grain prices, thus making feeding DDGS to livestock economically attractive (Dugan et al., 2010). While considerable research has been conducted on the impact of feeding DDGS on the quality of beef *longissimus* muscle, little research has been conducted on the impact of feeding cattle DDGS on the quality of other primal cuts, such as the *semimembranosus.* Therefore, the objective of

this study was to compare the meat quality traits of raw and pre-cooked beef roasts prepared from the *semimembranosus* muscle of crossbred beef steers fed wheat, corn and wheat/corn DDGS.

4.3 Materials and Methods

4.3.1 Animal Management and Diet Composition

This trial commenced in November 2008, at the University of Saskatchewan—Beef Cattle Research Station (Saskatoon, SK, Canada). In total, 288 commercial crossbred steers were purchased and subjected to a 70-day backgrounding period followed by a finishing period. The steers were implanted at the start of the test with Synovex S® (Pfizer Canada, Inc., Kirkland, QC) and re-implanted with Synovex Plus (Pfizer Canada, Inc., Kirkland, QC) at the start of the finishing period. The cattle were then randomly assigned to one of 24 pens and each pen was assigned to one of four dietary treatments. The control diet was a standard barley-based finishing diet comprised of 87.8% barley, 6.5% barley silage, and 5.7% supplement (DM basis). The other diets involved substitution of wheat, corn, or blend DDGS for barley grain in the control diet at 40% of the dietary DM. The wheat DDGS was supplied by Terra Grain Fuels (Belle Plaine, SK), the corn DDGS was purchased in two loads from ConAgra Foods (Omaha, NE) and from Blue Flint Ethanol (Underwood, ND). The blend DDGS was supplied by Husky Energy Inc. (Lloydminister, SK) and was the by-product of an ethanol fermentation run that used a 50:50 wheat and corn grain mix.

From the 288 steers, 80 (20 per treatment) were selected for in-depth meat quality analysis. Five steers from each of the four treatments were selected on the basis of live weight (625 kg target) on four separate slaughter dates. The selected steers at each slaughter date were loaded as a group (n = 20) at the University of Saskatchewan—Beef Cattle Research Station and transported (approximately 6 h) to Plains Processing Ltd. (PPL; Carman, MB, Canada). Upon receipt at PPL, the animals were held in lairage overnight with free access to water. The steers were slaughtered the following morning at the PPL abattoir, with all animals being held throughout the study under the guidelines established by the Canadian Council of Animal Care (1993).

4.3.2 Carcass Handling and Sample Collection

Following slaughter, the carcasses were split and chilled at 2°C (wind speed of 0.5 m/s) for 24 h. At 24 h, the left carcass sides were knife-ribbed at the Canadian grade site (between the 12th and 13th ribs) and exposed to atmospheric oxygen for twenty minutes. Following grading, carcass sides were quartered and loaded onto a refrigerated trailer for transport (approximately 20 min) to Keystone Processors Ltd. (KPL; Winnipeg, MB, Canada). Carcass quarters were refrigerated (4°C).

At 3 d postmortem (PM), the left and right inside rounds were removed from each carcass. The inside rounds were labeled, vacuum-packaged, palletized, and stored (4°C) at KPL until delivery to the University of Saskatchewan. Six days PM, the pallets were loaded onto a refrigerated trailer and transported (approximately 6 h) to the University of Saskatchewan. Upon arrival at the University of Saskatchewan, the inside rounds were transferred to the refrigerated meat processing room (4°C) located in the University of Saskatchewan's Department of Food and Bioproduct Sciences Pilot Plant.

Six days PM, the *semimembranosus* muscle was isolated through removal of the adductor and gracilus muscles, as well as all visible fat and connective tissue, before an initial pH reading was obtained by inserting a Hanna Electrode pH Meter (Hanna H2 9025 Microcomputer pH Meter; Hanna Instruments, Woonsocket, RI) into the *semimembranosus* muscle. The denuded *semimembranosus* muscles were again vacuum packaged into standard 3mm vacuum-packages, palletized, and placed in cold storage (-1°C) until 14 d PM. Fourteen days PM, the pallets were transferred to frozen (-30°C) storage for one year.

4.3.3 Preparation of Beef Roasts

All processing was carried out in the refrigerated meat processing room (<7°C) at the University of Saskatchewan (Saskatoon, SK). Forty-eight *semimembranosus* muscles (SM) (12 per treatment) from the left carcass side were randomly selected for value-added meat processing analysis on three different processing days (16 roasts per day). Roasts were removed from frozen storage and allowed to thaw (4°C) for 72 h. Twenty-four hours prior to processing, a brine was prepared to deliver 0.85% food-grade sodium chloride and 0.40% sodium tripolyphosphate (STTP; Newly Weds Foods Co.; Edmonton,

AB) into the finished product, achieving 20% extension by weight; however, there were higher cook losses than anticipated so actual levels in the meat were 0.97% sodium chloride and 0.46% STTP in the cooked product and 0.68% sodium chloride and 0.32% STTP in the raw product. The brine was then placed into cold storage (-1°C) until processing.

Each SM was divided into four pieces **(Figure 4-1)**. Section 1 was designated for cooked injected roast analysis and sections 2 and 4 were designated for raw non-injected or raw injected roast analysis. Sections 2 and 4 were balanced to ensure location within muscle was evenly represented in the trial.

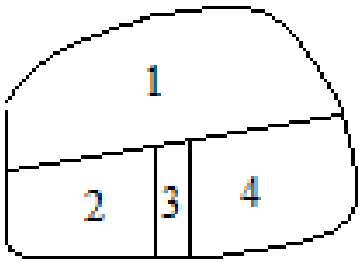

Figure 4-1 Location of roasts removed from the inside round. Section 1 designated for pre-cooked roast analysis, section 2 and 4 were designated for raw roast analysis, and section 3 was designated for colour analysis.

One steak (2.0 cm thick) designated for colour analysis was removed from the inside round (section 3). The steak was placed onto a Styrofoam tray with a soaker pad (Ultra Zap 1SW 1SPPERF Absorbent Soaker Pad; Paper Pak Industries; Washington, GA), over-wrapped with an oxygen permeable film (108 cc m^2 24 h^1 Huntsman choice wrap; Huntsman Packaging Corporation; Uniontown, OH) and placed in the meat processing room to bloom (3 h).

4.3.3.1 Raw Beef Roasts

Initial roast weights were obtained before the sections designated for raw injected analysis was subjected to brine injection using a multi-needle injector (Model No. FGM20/40; Fomaco Reiser; Burlington, ON). These roasts were then re-weighed to determine the pump percentage (i.e. injected weight / raw weight x 100) and placed into vacuum-package bags. Those roasts which did not reach their target weight were further

injected with the marinade using a hand injector to make up the difference. These roasts were vacuum-packaged and were intermittently tumbled (8.5 rpm) for a total of 2 h (20 min on, 10 min off) at 4-6°C in a VarioVac tumbler (Model #VV1-150; Killvoangen, Germany). Following tumbling, the roasts were placed in dark storage (4°C) for the brine to equilibrate.

Twenty-four hours after injection, the raw injected roasts were re-weighed to determine the percentage brine pickup (e.g., final weight / raw weight x 100). A steak was then removed from the injected roast. This steak was ground using an Osterizer 10 Speed Blender (Oster; Mexico City, Mexico) for subsequent TBARS analysis. The pH of the raw injected SM was also measured on the freshly ground beef using the modified slurry pH method. The remaining meat was placed into sterile sampling bags and stored under dark conditions (4°C) for subsequent TBARS analysis on days 1 and 7.

Likewise, a steak from the non-injected roast was removed for TBARS and proximate analysis. This piece was placed in a sterile sampling bag, with the remaining non-injected roast being vacuum-packaged and placed in dark storage (4°C).

The raw non-injected steak was ground using an Osterizer Blender for subsequent TBARS and proximate analysis. Moisture and slurry pH analysis was done on the freshly ground meat, while the remaining meat was placed into a sterile sampling bag and stored under dark conditions for subsequent TBARS analysis on days 1 and 7. Following TBARS analysis, the meat was frozen (-30°C) for subsequent fat analysis.

Lastly, the raw non-injected roasts were water cooked (2.5 h) in an air-agitated waterbath at 75°C to a final internal temperature of 72°C. After the roasts were cooked, they were cooled in an ice bath (1 h). Following cooling, the roasts were re-weighed to determine cook yield; a steak (2.5 cm) was removed for instrumental shear analysis in order to get a baseline shear value of the roasts prior to injection.

4.3.3.2 Cooked Beef Roasts

Following injection of the sections designated for raw SM analysis, the sections designated for cooked roast analysis underwent injection. The SM halves for cooked roast analysis were weighed before and after injection to determine the pump percentage, with the roasts not reaching their target weight being further injected with the marinade

using a hand injector to make up the difference. These roasts were vacuum-packaged and intermittently tumbled (8.5 rpm) for a total of 2 h (20 min on, 10 min off) at 4-6°C in a VarioVac tumbler (Model #VV1-150; Killvoangen, Germany). Following tumbling, the roasts were placed in dark storage (4°C) for the brine to equilibrate.

Thirty-six hours after injection, the injected SM halves were water cooked (4.5 h) in an air-agitated water bath at 75°C to a final internal temperature of 72°C following the heating curve outlined in **Figure 4-2**. After the roasts were cooked, they were cooled in an ice bath (2 h). Following cooling, the roasts were re-weighed to determine yield over green weight (e.g. cooked weight / raw non-injected weight x 100) and cook yield (e.g. cooked weight / raw injected weight x 100). Each roast was then divided into three sections for TBARS, expressible moisture, pH, and instrumental tenderness evaluation on days 1, 28 and 56. Locations within this half were balanced to ensure each section was evenly represented at each sampling day of the trial.

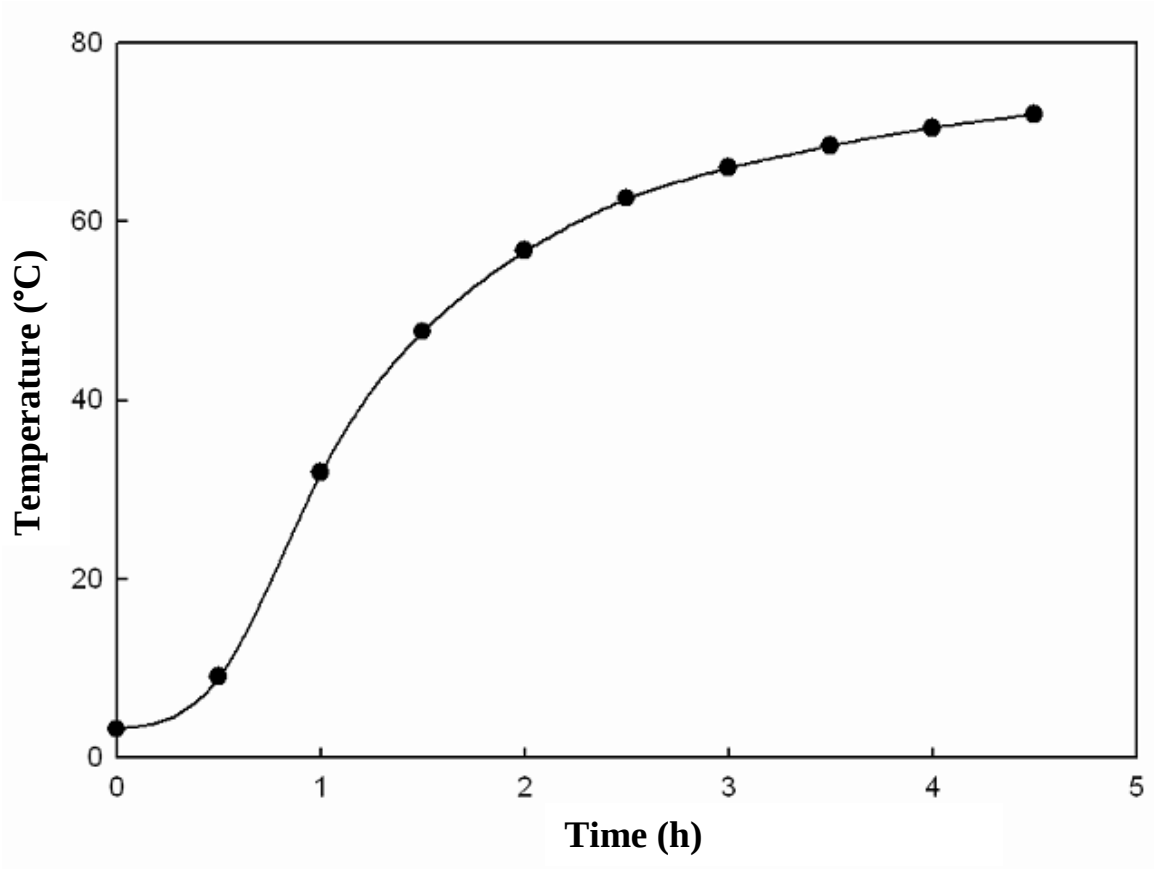

Figure 4-2 Standard heating curve for injected SM roasts cooked in an air-agitated water bath at 75°C.

The roasts designated for day 28 and 56 analysis were then weighed, vacuum-packaged, and placed in dark storage (4°C). For the roasts designated for day 1 analysis, a steak (2.5 cm) was removed from the centre for instrumental shear evaluation. Following removal of the steak, a slice of roast (8 mm) was removed using a Shillington Enterprises deli slicer (Shillington, PA) for expressible moisture analysis. From the remaining roast, the outside edges were trimmed and the meat was ground using an Osterizer Blender for pH, TBARS and moisture analysis; while proximate analysis was conducted only on the roasts designated for day 1 analysis. Following TBARS analysis, the ground meat used for day 1 cooked roast analysis was frozen (-30°C) for subsequent fat and protein analysis. The same procedure was carried out for the roasts designated for day 28 and 56 analysis; however, the roasts were weighed before cutting to determine purge loss (e.g. cooked weight after storage / cooked weight prior to storage x 100).

4.3.4 Objective Colour Evaluation

Following blooming, objective colour measurements were made on the steak designated for colour analysis, in duplicate, from the centre of each steak (Hunter *L** (lightness), *a** (red-green axis), *b** (yellow-blue axis); Commission Internationale de l'Eclairage, 1978) using the Hunter Lab Miniscan 45/0-L XE (Hunter Association Laboratory Inc.; Reston, VA) equipped with Illuminant A and Observer 10 settings. The steaks were then placed in a retail display case (4°C; Hussmann SSM-6; Hussmann Corporation; Gloversville, NY) equipped with deluxe warm-white fluorescent lighting (F32T8/TL741 Fluorescent Bulb; Philips Inc.; Somerset, NJ) adjusted to deliver 1210 lx to assess retail storage life. Steaks were objectively evaluated, as previously described, every 24 h for 4 d.

4.3.5 pH and Proximate Analysis

Approximately 4 g fresh meat was weighed, in duplicate, into pre-dried aluminum pans and analyzed for moisture content (Isotemp Oven Model 750F; Fischer Scientific; Singapore) using method 950.46 (AOAC, 1990). The pH was also measured on the freshly ground beef using a modified slurry pH method outlined in the *Handbook of Meat Analysis* (1985). In duplicate, 20 g of meat was placed in a stomacher bag and diluted

with 80 mL distilled water. The sample was stomached for 3 min before the pH was read by swirling the electrode from a pH meter (Accumet Basic AB 15 Plus pH Meter; Fischer Scientific; Singapore) into the slurry. Crude fat content was analyzed by petroleum ether extraction (Labconco Goldfisch Apparatus; Labconco Corporation; Kansas City, MI) using method 960.39 (AOAC, 1990); nitrogen content was determined by digestion (Buchi K435KB Digestion Unit; Buchi Labortechnik AG; Flawil, Switzerland) and distillation (Buchi B324 Distillation Unit; Buchi Labortechnik AG; Flawill, Switzerland) using method 981.10 (AOAC, 1990) using a protein conversion factor of 6.25.

4.3.6 TBARS Analysis

TBARS analysis occurred on days 1 and 7 for the raw roast samples, and on days 1, 28 and 56 for the cooked roast samples, as described in section 4.3.3.2, according to a modified method of Witte et al. (1970) as reported by Bedinghaus and Ockerman (1995). TBARS values (mg malondialdehyde per kg meat) were obtained by multiplying the absorbance of each sample by a constant (K) (Witte et al., 1970), whose formula was modified from Tarladgis et al. (1960), which takes into account the slope of the standard curve and the percent recovery of the system. A K-value of 21.61 was calculated from a slope of 1.59×10^7 and an 84.00% recovery of TMP for both the raw and cooked product.

4.3.7 Instrumental Shear Analysis

The Warner-Bratzler shear force (WBSF) of 6-8 core samples (2.5 cm x 1.2 cm x 1.2 cm) cut parallel to the fibre direction from each cooked roast was determined. Following removal of the steak from the roast, cores were removed and placed into polyethylene bags, sealed, and transferred to a 4°C cooler. The following morning, the cores were removed from cold storage and allowed to equilibrate to room temperature. Peak shear force was determined on each core perpendicular to the fibre grain using a TMS Pro Texture Press equipped with a Warner-Bratzler shear head at a crosshead speed of 200 mm/min and 30 kg load cell using Texture Lab Pro v. 1.12 Software (Food Technology Corporation; Sterling, VA), with the average peak shear force being reported for each roast.

Force-deformation curves from the Warner-Bratzler shear device were also used to determine WBSF myofibrillar component (SF-M) and WBSF connective tissue component (SF-C), with the initial yield (height of the first peak) corresponding to SF-M and the final yield (height of the second peak) corresponding to SF-C (Pietrasik et al., 2010). Both of these were measured from the shear curves, as suggested by Møller (1981), with slight modifications. Specifically, SF-M force was measured on the shear deformation curve as a peak value occurring within the first half of the distance the shear blade cut through the meat sample; while the SF-C was measured as a peak value within the last 3 mm before the shear blade had completed cutting through the meat sample (Pietrasik et al., 2010). Maximum peak force recorded during the test was reported as WBSF.

4.3.8 Expressible Moisture

Expressible moisture (EM) was determined by a modified method of Foegeding and Ramsey (1986) as reported by Jaurequi et al. (1998). Specifically, three meat samples of known weight (1.5 ± 0.3 g) were removed from each roast beef slice using a #9 cork borer. Each sample was then placed into a 50-mL centrifuge tube fitted with a thimble consisting of Whatman No. 3 filter paper folded around Whatman No. 50 filter paper. The samples were centrifuged for 15 min at 750 x *g* using a Thermofisher Scientific Sorvall RC-6 Plus Centrifuge (Asheville, NC). EM was expressed as the percentage of weight lost after centrifugation to the initial sample weight.

4.3.9 Statistical Analysis

Data were analyzed using PROC MIXED option of SAS 9.2. Colour, TBARS, WBSF, EM and cooked pH data were analyzed using repeated measures, with diet and day as the main effects and day as the repeated measure using an ante-dependence covariance model. Processing trial and section were included as random effects, while section was also included as a covariate. The remaining data were analyzed as a one-way ANOVA, using diet as the main effect and processing trial as a random effect. Means were separated using PDIFF procedure and Tukey-Kramer Highest Significant Difference. Significance and trends were declared at $P \leq 0.05$ and 0.10, respectively.

4.4 Results and Discussion

4.4.1 Meat Quality

In general, the type of diet had no effect ($P>0.05$) on the chemical composition of raw or cooked SM roasts obtained from steers fed various DDGS diets **(Table 4-1)**. These findings are in agreement with Adam et al. (2010), who did not observe a difference in the chemical composition of SM muscles obtained from Nilotic kids fed sorghum and molasses-based diets. However, differences were observed in the chemical composition of raw versus cooked meat. Specifically, raw non-injected beef roasts had a higher moisture and fat content than the cooked injected beef roasts. Similar results were obtained by Dhanda et al. (2002), who found that injected bison SM had lower protein and fat contents compared to non-injected samples.

When compared to the *longissimus* muscle analyzed in Chapter 3, some slight muscle differences were observed. In particular, the *longissimus* muscle had slightly higher moisture (72.97% versus 70.78%) levels than the SM but slightly lower levels of protein (22.33% versus 24.57%) and fat (3.38% versus 3.65%). These findings contrast those of Lorenzen et al. (2007), who found that both the longissimus and SM had a similar moisture content (72.8% and 72.6%, respectively) and a slightly higher fat content (4.2% and 3.8%, respectively) than the present study. Some of these differences can be attributed to variations in muscle composition but differences may have arisen as a result of differences in handling between the muscles. Whereas the SM was subjected to a year of frozen storage prior to analysis, the meat used for proximate analysis on the *longissimus* muscle was not frozen prior to sample preparation. Therefore, there wasn't moisture loss from the *longissimus* samples in the form of drip loss that may have impacted meat composition. In contrast to the *longissimus* muscle, thawing of the SM after frozen storage resulted in moisture loss from the product. Because this moisture was poured off and not accounted for during analysis, the proximate composition of the SM underestimated the moisture content and, as a result, overestimated the protein and fat content.

Table 4-1 Effect of diet (treatment) on meat quality attributes of raw and cooked *semimembranosus* roasts obtained from steers (n=48) fed various DDGS diets.

	Treatment				SEM	Day			SEM*	*P*-value		
		DDGS Diet										
	Control	Corn	Wheat	Blend		1	28	56		Diet	Day	Diet x Day
Raw Roast												
Moisture (%)	70.63	70.62	70.93	70.95	0.317	n/a	n/a	n/a	n/a	0.799	n/a	n/a
Fat (%)	3.91	3.67	4.01	3.01	0.481	n/a	n/a	n/a	n/a	0.465	n/a	n/a
Non-Injected pH	5.41	5.43	5.40	5.40	0.021	n/a	n/a	n/a	n/a	0.631	n/a	n/a
Injected pH	5.67	5.70	5.71	5.68	0.030	n/a	n/a	n/a	n/a	0.754	n/a	n/a
Cooked Injected Roast												
Moisture (%)	69.11	68.36	69.20	69.69	0.526	n/a	n/a	n/a	n/a	0.175	n/a	n/a
Protein (%)	26.34	26.73	26.19	25.59	0.544	n/a	n/a	n/a	n/a	0.256	n/a	n/a
Fat (%)	2.77	2.97	2.92	2.83	0.369	n/a	n/a	n/a	n/a	0.983	n/a	n/a
Injected pH	5.90	5.91	5.92	5.89	0.017	5.96	5.93	5.82	0.015	0.697	<0.001	0.994

* Pooled SEM

n/a = not applicable

Injection of the salt/phosphate brine also influenced the proximate composition of the SM. Although proximate analysis was not conducted on the raw injected SM roasts, the moisture, protein and fat levels were mathematically calculated based on the injection of 20 additional parts of moisture into the SM. Because of the addition of 20% brine into the SM, the moisture content increased to 74.26%, while the protein content decreased to 20.48% and the fat content decreased to 3.26%.

When comparing raw versus cooked samples, it is typically assumed that cooking will lead to moisture loss via cook loss, concentrating the protein and fat portions, increasing their concentrations. However, in this experiment the roasts designated for cooking were first injected with a salt/phosphate brine. The inclusion of extra water into the meat matrix compensated for the moisture loss, which can explain why there was not a notable difference in moisture content between raw and cooked meat.

However, not all of the differences can be attributed to brine injection. During the course of the experiment, one half of the SM was consistently used to represent the raw meat while the other half was consistently used to represent cooked meat. In the cooked meat, it was observed that a major fat seam ran through one of the sections; therefore, one section had a considerably higher fat content than the other sections. Interestingly, the section in the cooked roast half containing the fat seam adjoined the area in the raw roast half where the samples were taken for proximate analysis. Because the locations within the SM half designated for cooked roast analysis were balanced to ensure each section was evenly represented in the trial, the lower fat levels of the two outside sections may have reduced the total fat levels of the cooked meat. This combination of brine injection and roast location may explain why fat levels in the cooked meat were significantly lower than those in the raw meat.

As in chemical composition, although no dietary differences were detected for pH, there were differences among the roast treatments. Specifically, cooked roasts had a higher pH than raw injected roasts, which, in turn, had a higher pH than raw non-injected roasts. It is well known that one of phosphate's many functions is to help increase cooking yields by increasing the pH and ionic strength (Trout and Schmidt, 1984), explaining why the samples injected with phosphate had higher pH values than the non-injected samples. Likewise, cooking increases hydrogen molecule concentrations present

in meat samples (Madruga and Mottram, 1995). Because pH is directly related to hydrogen molecule concentration, an increase in hydrogen molecules will result in an increase in pH, as is demonstrated when comparing raw injected roasts to cooked roasts.

A day effect was also observed for the pH of the cooked roast samples. For the injected roasts, the pH decreased from 5.96 to 5.82 over time in the vacuum-packaged system ($P<0.001$). It has been shown that lactic acid bacteria are the predominating flora in vacuum and low oxygen atmospheres (Borch and Molin, 1988). At the present storage temperature (4°C), it is hypothesized that the pH of cooked meat decreased with time due to the formation of lactic acid from bacterial activity (Pietrasik et al., 2006). Pietrasik et al. (2006) and Jeremiah and Gibson (2001) also reported a pH decrease in vacuum-packaged cooked beef during storage at 4°C.

4.4.2 Lipid Oxidation in Raw SM Roasts

The thiobarbituric acid test is one of the most widely used methods for measuring the extent of lipid oxidation in meat products (Pietrasik et al., 2006). In this procedure, malondialdehyde reacts with the TBA reagent to form a pink-coloured chromogen, which is measured using a spectrophotometer; however, other secondary products of lipid oxidation can also react with the reagent so the name TBARS is used (Pietrasik et al., 2006) with the RS indicating "reactive substances".

Since lipid oxidation occurs at a slower rate than discolouration in fresh meat, it is not normally considered to be a limiting factor for the shelf-life of aerobically-packed chill stored meat (Zhao et al., 1994), but lipid oxidation can influence colour and colour stability. This occurs because there is a direct relationship between pigment and lipid oxidation, with the meat that has the poorest oxidative stability having the worst colour stability (Pietrasik et al., 2006). This occurs because muscles with the poorest colour stability were observed to have the greatest oxidative activity and the highest rates of myoglobin autoxidation (Renerre and Labas, 1987).

In the current study, both diet and day had a significant ($P<0.001$) effect on the oxidative stability of non-injected SM roasts **(Table 4-2)**. Specifically, raw SM obtained from steers fed a DDGS diet displayed a 50% increase in TBARS values compared to raw SM obtained from steers fed the control diet. More importantly, mean TBARS values

exceeded the acceptable threshold value (2.0 mg malondialdehyde per kg meat; Campo et al., 2006) where rancid off-flavours are first perceived by trained sensory panelists. Similar results were obtained by Koger et al. (2010), who observed ground beef from steers fed 40% corn DDGS had increased TBARS after 2 d storage when compared to ground beef from steers fed a corn control diet. This result was expected because meat obtained from steers fed DDGS have elevated levels of unsaturated fatty acids (Chapter 3; Aldai et al., 2010a; Dugan et al., 2010). These unsaturated fatty acids react with oxygen, starting the chain of lipid oxidation that can lead to rancidity, off-flavours, and meat discolouration.

Table 4-2 TBARS values of raw non-injected and injected *semimembranosus* roasts obtained from steers (n=48) fed various DDGS diets.

	Treatment				SEM	Day		SEM*	*P*-value		
		DDGS Diet									
	Control	Corn	Wheat	Blend		1	7		Diet	Day	Diet x Day
TBARS [1]											
Non-Injected	2.23[b]	3.32[a]	3.34[a]	3.72[a]	0.218	1.38	4.93	0.126	<0.001	<0.001	<0.001
Injected	1.60	1.98	1.95	2.09	0.138	0.54	3.28	0.091	0.070	<0.001	0.022

* Pooled SEM

[1] mg malondialdehyde per kilogram of beef

High TBARS values are of concern because high TBARS values reduce consumer acceptance of cooked products. In comparison to the previous study, which evaluated the effect of DDGS diet on the oxidative stability of raw and cooked *longissimus* muscle, the average TBARS values are much lower in the SM as compared to the raw *longissimus* muscle (3.15 and 4.96, respectively). Prior to analysis, the SM were placed in frozen (-30°C) storage for one year, whereas the *longissimus* muscle, which were not subjected to a freeze-thaw cycle, was aged 14 d and immediately analysed. Hansen et al. (2004) observed that a freeze-thaw cycle stimulated the onset of lipid oxidation in pork chops. However, the results in the current study contrast the findings of Hansen et al. (2004) due to the fact that the overall TBARS values of the frozen meat were lower than the overall TBARS values of the fresh meat. The lower TBARS values in the SM muscle can be explained by limitations in the TBARS test.

Specifically, malondialdehyde and other secondary oxidation products normally detected in the TBARS test are not stable for long periods of time because they are further oxidized to produce organic alcohols and acids that are not detected in the TBARS test (Fernandez et al., 1997). Therefore, it is possible that some of the secondary oxidation products present in the SM underwent further oxidation, resulting in overall lower TBARS values.

In another study, Lorenzen et al. (2007) found the *longissimus lumborum* muscle to have an elevated fat content compared to the SM (4.2 and 3.8%, respectively) so it is possible that the elevated fat content resulted in increased levels of lipid oxidation in the *longissimus* muscle. When comparing the fat contents of the muscles used in analysis, the fat content in the *longissimus* muscle was lower than the fat content in the SM (3.4 and 3.7%, respectively); therefore, this hypothesis does not hold true in the present study.

Differences in oxidative stability between *longissimus* muscle and SM can be explained by differences in the fatty acid profile between the two muscles. Lorenzen et al. (2007) examined the effect of feeding grass versus grain diets on the fatty acid composition of various bovine muscles, including the *longissimus lumborum* and the SM. They discovered that, although the two muscles did not differ in total polyunsaturated fatty acid content, the *longissimus lumborum* contained elevated levels of total unsaturated fatty acids when compared to the SM (427.73 and 404.12 g per mg fat, respectively). Therefore, it is possible that in the current study, elevated levels of unsaturated fatty acids in the *longissimus* muscle resulted in elevated TBARS values due to an increased amount of lipid oxidation.

Not only did diet and day have an effect on TBARS values of the raw non-injected SM roasts, but a diet by day interaction was also detected **(Figure 4-3)** as a result of the average TBARS values of the ground meat obtained from the steers fed a DDGS diet diverging from the average TBARS value of the ground meat obtained from the steers fed the control diet. As expected, SM obtained from steers fed the control diet had significantly lower TBARS values than SM obtained from steers fed blend DDGS after 1 d storage. Similarly, after 7 d of dark storage (4°C), SM obtained from steers fed the control diet had significantly lower TBARS values than SM obtained from steers fed a

DDGS diet due to the meat obtained from steers fed a DDGS diet having higher levels of unsaturated fatty acids than the control meat.

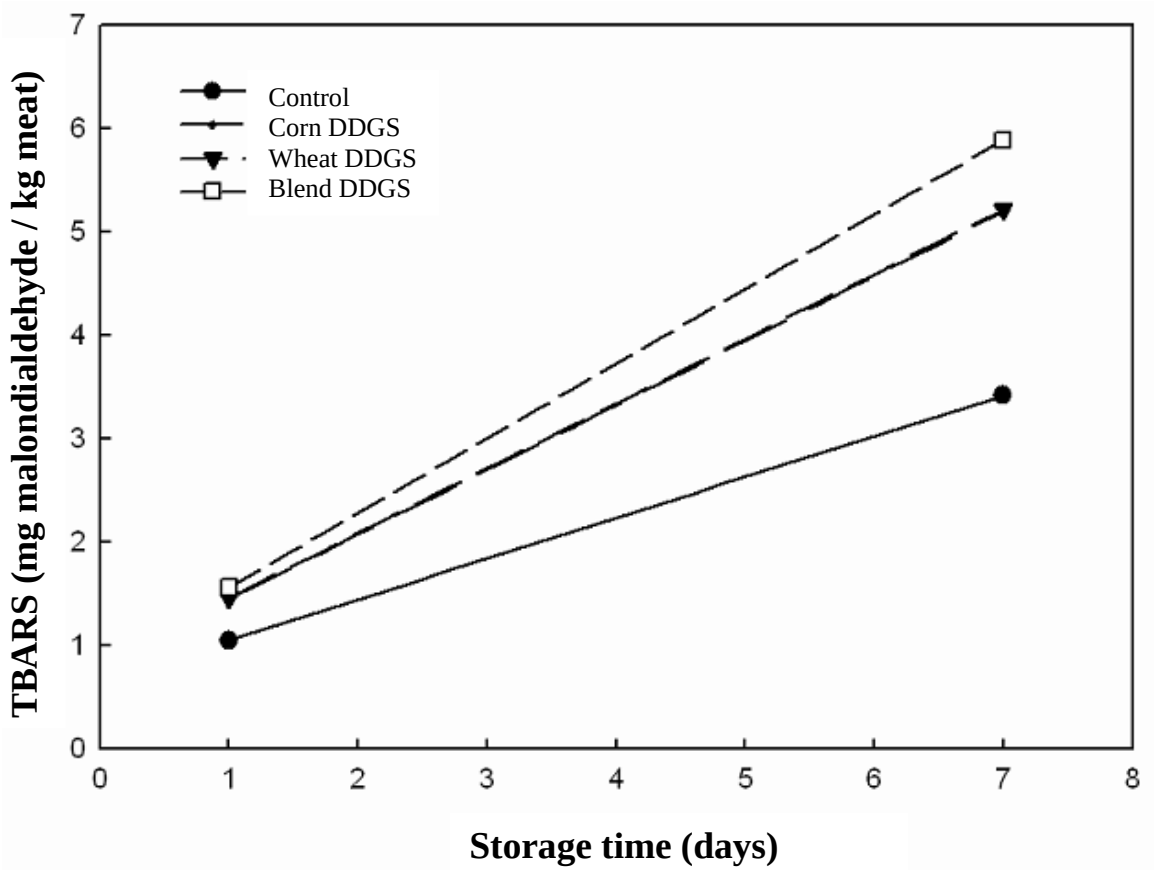

Figure 4-3 Effects of feeding the control or wheat, corn or blend dried distillers grains plus solubles (DDGS) diets to crossbred beef steers on the oxidative stability of raw ground non-injected *semimembranosus* meat after 7 days of storage (4°C; n=48). Corn DDGS diet is hidden by wheat DDGS diet.

Similar to what was observed with the raw non-injected SM roasts, meat obtained from steers fed a DDGS diet tended (P=0.07) to have higher TBARS values than meat obtained from steers fed the control diet. Any dietary effect observed in raw non-injected SM was lessened by the addition of salt and phosphate in the brine. This is evident, with a 65% reduction in TBARS values between the raw non-injected roasts and the raw injected roasts. The addition of salt into a meat matrix is essential for achieving microbiological stability by decreasing water activity, for making possible the

solubilization of muscle proteins which allows for gel formation and the development of an optimum texture, and for imparting a salty taste in the final product (Martin, 2001); however, sodium chloride also has a prooxidant effect (Min et al., 2010). It works to promote oxidation by: (1) disrupting the structural integrity of cell membranes, which enables catalysts easy access to lipid substrates (Rhee, 1999); (2) releasing free ionic iron from iron-containing molecules such as heme proteins (Rhee and Ziprin, 2001); and (3) inhibiting the activities of antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (Lee et al., 1997).

Even though salt, a prooxidant, was added into the meat, the overall TBARS values from the raw injected SM roasts were lower than the overall TBARS values from the raw non-injected SM roasts. These lower TBARS values can be attributed to the addition of sodium tripolyphosphate into the meat. Phosphates are typically used as a basic ingredient in brines to increase cooking yield by increasing pH and ionic strength; however, phosphates also act to chelate ions that act as catalysts for oxidation, such as iron (Cheng and Ockerman, 2003). This hypothesis is supported by research from Cheng and Ockerman (2007), who found that the addition of salt into pre-cooked beef roasts did not promote lipid oxidation due to the benefits of the chelating agent (phosphate) balancing the disadvantages of the salt's prooxidant effect.

A diet by day interaction for TBARS was also detected for the raw injected SM roasts **(Figure 4-4)**. These results show that TBARS values of SM roasts obtained from steers fed the control diet were diverging from TBARS values of SM roasts obtained from steers fed a DDGS diet by the last day of storage. In total, the average TBARS values of raw injected meat obtained from steers fed a DDGS diet were 24% higher than TBARS values of raw injected meat obtained from steers fed the control diet. Similar to observations in the raw non-injected SM roasts, the diverging TBARS values in the SM roasts obtained from steers fed a DDGS diet can be attributed to higher levels of unsaturated fatty acids in the meat obtained from steers fed a DDGS diet. Because of these higher levels of unsaturated fatty acids, meat obtained from steers fed a DDGS diet will more readily undergo oxidation, resulting in overall higher TBARS values.

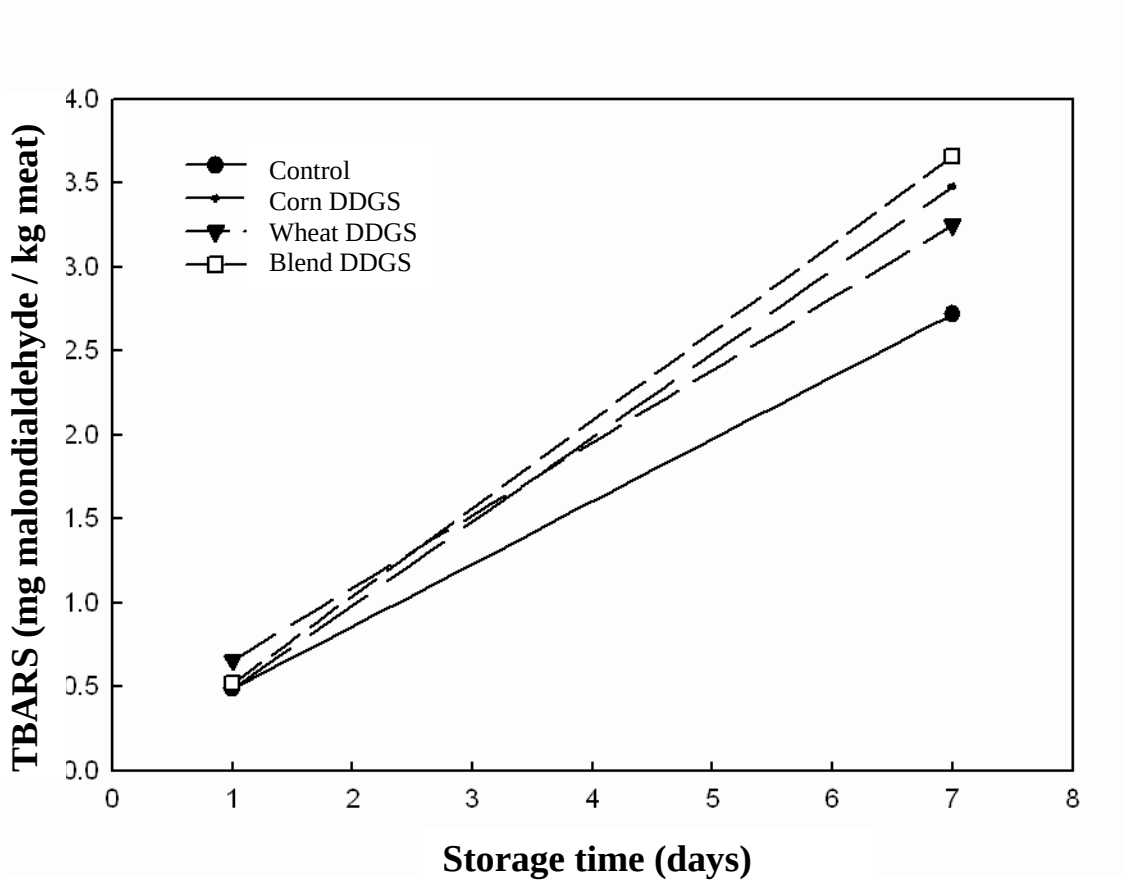

Figure 4-4 Effects of feeding the control or wheat, corn or blend dried distillers grains plus solubles (DDGS) diets to crossbred beef steers on the oxidative stability of raw ground *semimembranosus* meat injected with a salt/phosphate brine after 7 days of storage (4°C; n=48).

4.4.3 Colour Evaluation

Overall, diet did not have an effect on L* or b* values **(Table 4-3).** These findings contrast previous work conducted on the *longissimus* muscle, where steaks obtained from steers fed wheat DDGS were lighter (higher L* value) than steaks obtained from steers fed other DDGS diets (Chapter 3). The lightness (L* value) and yellowness (b* value) are generally not considered appropriate for assessing colour stability (Dunne et al., 2005) because it was reported that they did not change during 10 days of retail display (Sapp et al., 1999). Instead, muscle redness has been extensively used as an index of discolouration in displayed beef (Yang et al., 2002). Muscles may also differ in their inherent colour stability, which is their intrinsic ability to resist metmyoglobin formation (Ledward et al., 1986). Using the a* value, it was found that the *longissimus* muscle is more colour stable than the *semimembranosus* muscle (Dunne et al., 2005).

Table 4-3 Effect of diet on Hunter colour measurements of *semimembranosus* steaks[1] obtained from steers fed various DDGS diets when stored under retail display conditions (4°C; 4 d).

	Treatment				SEM	Day					SEM[2]	*P*-value		
		DDGS Diet												
	Control	Corn	Wheat	Blend		0	1	2	3	4		Diet	Day	Diet x Day
L*[3]	40.19	40.61	40.97	38.87	1.03	42.44	40.94	39.71	39.09	38.60	0.531	0.500	<0.001	0.367
a*[4]	23.27[a]	21.93[b]	21.70[b]	21.03[b]	0.440	30.57	24.78	22.33	17.29	14.94	0.310	0.007	<0.001	0.050
b*[5]	20.25	20.06	20.06	19.93	0.482	25.42	21.17	19.52	17.54	16.72	0.317	0.973	<0.001	0.662

[1] 12 steaks / treatment.

[2] Pooled SEM

[3] L* lightness (0 = black, 100 = white)

[4] a* redness (positive values = red, negative values = green)

[5] b* yellowness (positive values = yellow, negative values = blue)

In the present study, SM steaks obtained from steers fed the control diet were better able to maintain redness over 4 d of retail display than SM steaks obtained from steers fed a DDGS diet. The poor colour stability of SM steaks, in general, can be attributed to a combination of the extrinsic and intrinsic factors; however, the accelerated colour deterioration in steaks obtained from steers fed DDGS is most likely related to the higher TBARS values observed in the raw non-injected meat obtained from steers fed a DDGS diet. This is because lipid oxidation in red meats compromises colour as well as flavour, with the reactive lipid oxidation products accelerating the formation of metmyoglobin (Lynch et al., 2001).

Likewise, a diet by day interaction was observed for the a* colour parameter **(Figure 4-5)**. There were no differences in redness until day 2, when the steaks obtained from steers fed DDGS were not as red as steaks obtained from steers fed the barley control diet. This trend was observed throughout the remainder of retail display, with steaks obtained from steers fed the barley control diet retaining their colour better than the steaks obtained from steers fed a DDGS diet. Unlike what was observed in the *longissimus* muscle, no differentiation was observed in the colour deterioration of steaks obtained from steers fed a DDGS diet.

Even though the steaks underwent rapid colour deterioration, all of the redness values were still above a suggested acceptability threshold of 12, based on data from O'Sullivan et al. (2002). Coincidently, it was observed that a threshold value of 12 coincided with steaks that were the most preferred at 4 days of retail display (Dunne et al., 2005); however, this work was conducted on fresh meat—not meat placed under frozen storage for one year. Therefore, it is difficult to compare the redness values in the current study to those proposed by O'Sullivan et al. (2002) because, visually, the steaks used in the current study were extremely discoloured (personal observation) after 4 days of storage although the *a** values were still well above the proposed threshold value.

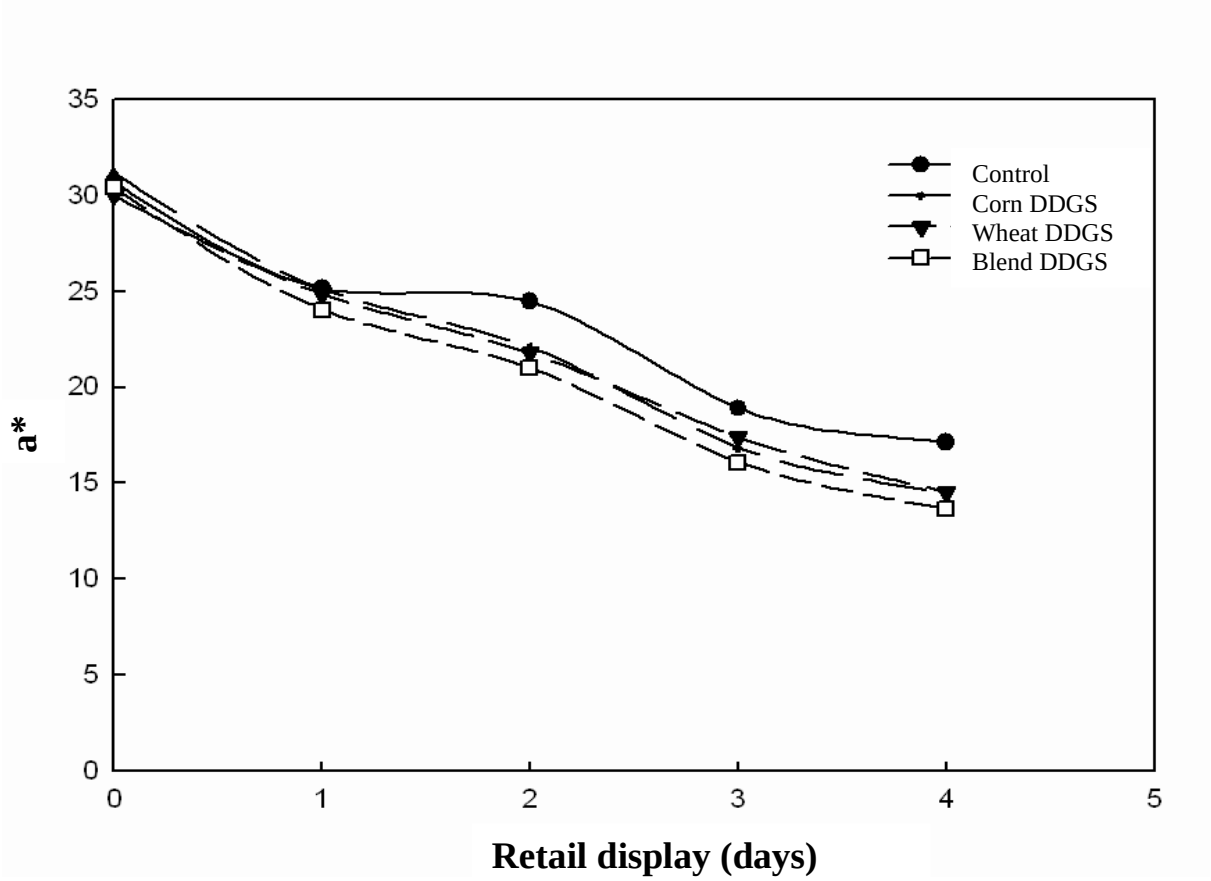

Figure 4-5 Effects of feeding the control or wheat, corn or blend dried distillers grains plus solubles (DDGS) diets to crossbred beef steers on a* (higher values = more red, lower values = more green) values of *semimembranosus* steaks after 4 days of simulated retail display (n=48).

The rate of colour loss in meat is dependent on a number of factors, including storage conditions (Renerre, 1990). Except for storage time, temperature is the most important extrinsic factor influencing the storage life of fresh meat (Jeyamkondan et al., 2000). Maximum storage life is achieved when meat is held at -1.5°C, which is the lowest temperature that can be maintained indefinitely without the muscle freezing (Gill and Jones, 1992). Increased storage temperature has a large impact on retail display life due to a more rapid rate of metmyoglobin formation at higher temperatures (Ledward et al., 1986). It has also been reported that a low storage temperature promotes oxygen penetration into meat surfaces, raising the solubility of oxygen in tissue fluids, which subsequently increases the depth of the oxymyoglobin layer on the meat surface (Hood, 1984).

Freezing and thawing meat can also have an impact on colour stability and has been reported to have adverse effects on the surface colour of beef (Lanari et al., 1993). Pietrasik and Janz (2009) found that freezing and thawing did not affect L* and b* values but they did find meat that had not been frozen was significantly redder (higher a* values) than thawed meat. Similarly, Boles and Swan (2002b) reported a decrease in redness after freezing *semimembranosus* muscles from Brahman cattle. Likewise, through studying the effects of various freeze-thaw cycles on the colour stability of *longissimus lumborum* muscles, Lanari et al. (1993) observed that freezing and thawing produced a considerable decrease in redness and saturation index. Jakobsson and Bengtsson (1973) hypothesized that the colour of beef after thawing was dependent on freezing rate, with meat frozen very slowly having significantly lower Hunter a* values compared to samples frozen more rapidly.

Storage conditions are not the only factor influencing the colour stability of various meat cuts; different muscles have unique purposes in living animals, resulting in variations in fibre type (Kirchofer et al., 2002) and metabolic function (Faustman and Cassens, 1991). This results in meats exhibiting a wide range of post-mortem oxygen consumption rates (OCR) and subsequent colour stabilities, depending on muscle source (Renerre and Labas, 1987) due to an inverse relationship between discolouration rate and OCR (Lanari and Cassens, 1991).

Differing colour stabilities of muscles can be attributed to differing muscle-fibre type and metabolic function (Seyfert et al., 2006). Muscle colour stability has been categorized into four groups on the basis of biochemical factors affecting meat discolouration: high colour stability (*longissimus lumborum, longissimus thoracis, semitendinosus* and *tensor faciae latae*); intermediate colour stability (*semimembranosus, rectus femoris, trapezius, gluteus medius* and *latissimus dorsi*); low colour stability (*biceps femoris, pectoralis profundus, adductor* and *triceps brachi*); and very low colour stability (*supraspinatus, infraspinatus* and *psoas major*) (McKenna et al., 2005). More specifically, muscles with greater oxidative metabolic activity have greater OCR and are less colour stable than those with glycolytic metabolic activity (Atkinson and Folett, 1973; O'Keefe and Hood, 1982). Metabolic enzymes consume oxygen post-mortem,

reducing the oxygen partial pressure at the meat surface, favouring the formation of deoxymyoglobin (Tang et al., 2005).

Poor colour stability of the SM can be attributed to locational differences within the muscle due to its size. Seyfort et al. (2006) observed that the deep SM (the inner region of the muscle closest to the femur) widely differed from the superficial SM (region of muscle adjacent to the subcutaneous fat) in colour stability. Several studies have reported higher colour intensity (chroma values) and a lighter (L*)- cherry red (a*) colour of the deep SM compared to the superficial SM at initial retail display (Sawyer et al., 2007; Gunderson et al., 2009); however, these studies also found rapid discolouration of the deep SM during display. Sammell et al. (2002) reported a lower OCR, metmyoglobin reducing activity, and colour stability of the deep SM compared to the superficial SM. Therefore, it is suggested that the two-toned colour formation and lower colour stability of the deep SM could be partially due to the modified redox chemistry of myoglobin caused by the high temperature and rapid pH decline during the cooling process rather than intrinsic factors, such as muscle fibre type, of the muscle (Kim et al., 2010). Consequently, the high temperature and rapid pH decline of the deep SM could negatively affect lactate dehydrogenase activity, and subsequently reduce metmyoglobin reducing ability (Kim et al., 2010). Kim et al. (2009) proposed the involvement of lactate dehydrogenase in metmyoglobin reduction and colour stability of bovine muscles after discovering that the colour stable *longissimus dorsi* muscle had higher lactate dehydrogenase activity, NADH and metmyoglobin reducing activity than the less stable *psoas major*, and inhibition of lactate dehydrogenase resulted in the decreased colour stability by decreasing the replenishment of NADH. Similarly, Zhu and Brewer (1998) found significantly lower metmyoglobin reducing activity in PSE compared to normal pork, suggesting that the high temperature/ low pH conditions encountered in PSE pork denatured the proteins involved in the metmyoglobin reducing system (Kim et al., 2010).

4.4.4 Processing Characteristics

Consistent with meat quality of the fresh meat, no dietary differences were observed in regards to the processing characteristics of the pre-cooked beef roasts **(Table 4-4)**. Differences in pump percentage or brine uptake were not expected because

processing conditions were established to ensure these parameters did not vary. Numerically, however, a large difference was observed in brine uptake, with roasts prepared from SM of steers fed the control diet having almost 6% higher brine uptake than roasts prepared from SM of steers fed corn DDGS.

Table 4-4 Effect of diet (treatment) on processing attributes of *semimembranosus* roasts obtained from steers (n=48) fed various DDGS diets.

	Treatment				SEM	Day		SEM	*P*-value		
		DDGS Diet									
	Control	Corn	Wheat	Blend		28	56		Diet	Day	Diet x Day
Pump (%)	20.02	19.97	19.99	19.97	0.029	n/a	n/a	n/a	0.677	n/a	n/a
Brine Uptake (%)	78.08	72.31	74.63	74.84	2.17*	n/a	n/a	n/a	0.275	n/a	n/a
Cook Yield (%)											
Non-Injected	72.70	72.56	72.18	73.39	0.660	n/a	n/a	n/a	0.630	n/a	n/a
Injected	71.16	70.81	70.47	71.18	0.956	n/a	n/a	n/a	0.695	n/a	n/a
Yield Over Green (%)											
Non-Injected	72.70	72.56	72.18	73.39	0.660	n/a	n/a	n/a	0.630	n/a	n/a
Injected	85.40	84.95	84.56	85.39	0.691	n/a	n/a	n/a	0.114	n/a	n/a
Purge Loss (%)	0.95	1.09	1.01	1.01	0.208	1.00	1.03	0.205*	0.584	0.619	0.825

* Pooled SEM

n/a = not applicable

Although steer selection was based on finishing weight, feeding the higher energy corn DDGS diet resulted in an improved feed to gain ratio, which results in less days on feed (Walter et al., 2010). This was confirmed by Amat et al. (2011, unpublished data), who found feeding the control, corn DDGS, wheat DDGS, and blend DDGS resulted in 216, 209, 218, and 205 days on feed, respectively. It is possible that the increased growth rate observed in steers fed corn DDGS resulted in the generation of larger SM when compared to steers fed the other finishing diets. The larger SM would have a slower chill rate and a more rapid pH decline compared to the SM of steers fed the other finishing diets, resulting in increased protein denaturation and a lower water-holding capacity. As a result of decreased protein functionality, the SM from steers fed corn DDGS wouldn't have the ability to hold as much water, resulting in numerically lower brine uptake. However, this hypothesis is not supported by overall cook yield values.

Similar pump percentage and brine uptake, differences were not observed in the cook yields of SM roasts obtained from steers fed various DDGS diets, which is in accordance with other findings of researchers examining the *longissimus* muscle (Leupp et al., 2009; Aldai et al., 2010b). This shows that feeding steers a DDGS diet has no effect on the water-holding capacity of the resulting roasts. However, the non-injected roasts had a higher cook yield than the injected roasts, which contrasts findings of previous researchers (Boles and Swan, 1997; Boles and Shand, 2001; McGee et al., 2003; Pietrasik et al., 2005). It is important to note that these studies did not report injected cook yield and yield over green. Instead, these studies reported yield over green as the cook yield. When evaluating overall cook yields, the yield over green weight for the non-injected roasts can be compared to the yield over green weight for the injected roasts because the yield over green weight for the non-injected roasts is equivalent to the cook yield. Keeping this in mind, the injected roasts having a higher yield over green than the non-injected roasts supports the findings of previous researchers (Boles and Swan, 1997; Boles and Shand, 2001; McGee et al., 2003; Pietrasik et al., 2005). Overall, very low cook yields were observed in this experiment, which may be attributed to a number of different factors.

Generally, it is accepted that injecting beef muscles with salt and phosphate solutions substantially improves the yield of cooked beef (Pietrasik and Shand, 2005) due, in part, to increased solubilization of the meat proteins and to a higher pH in the injected meat **(Table 4-1)**. There is a clear tendency for the water-holding capacities to increase with increasing pH (Trout and Schmidt, 1986). In meat systems, the water binding capacity is lowest at pH 5.4, which is the isoelectric point of actomyosin (Pietrasik and Janz, 2009); however, as the muscle pH is increased, thaw-exudate progressively decreases until a minimum purge is reached at pH 6.4 (Deatherage and Hamm, 1960). One important characteristic of phosphates is their ability to move meat pH away from the isoelectric range, increasing the proportion of negative charges on the meat proteins, thus improving water holding capacity and binding (Pietrasik and Janz, 2009). The increased water-holding capacity due to phosphates is caused by the unfolding of the three-dimensional protein network by the high ionic strength, causing the muscle to swell and the protein to solubilize and retain water before heating (Torley

et al., 2000). Furthermore, the influence of phosphates on improving water holding capacity is enhanced when used in combination with sodium chloride (Trout and Schmidt, 1986) because the addition of salt increases the ionic strength of the injected solution, thereby increasing the number of hydrophilic protein interactions within the meat and increasing the binding of free water (Pietrasik and Janz, 2009).

The relationship of salt to cook loss has been reported by numerous researchers for many types of processed meats. Baubits et al. (2006) reported a linear decrease in cooking losses in injected meats with NaCl levels increasing from 0.5 to 1.5%. Supporting the findings of Baubits et al. (2006), Shackelford et al. (1989) observed that tumbled chuck roasts displayed a significant yield increase as salt content was increased from 1 to 2%. Similarly, Trout and Schmidt (1984) observed increased cook yields by increasing NaCl levels from 0.95 to 1.65% while holding sodium tripolyphosphate levels constant, attributing the increased yields to increased ionic strength. In addition, the injected beef roasts underwent tumbling, which has been found to increase cook yields of pre-cooked beef roasts significantly by allowing for a more even distribution of salts and phosphates within the SM (Cheng and Ockerman, 2003). With this in mind, it can be seen that the increase in protein solubility as a result of salt and sodium tripolyphosphate addition helped hold onto an additional 12% water when compared to the non-injected samples.

Another important factor that may have influenced the overall processing characteristics is the fact that the SM used in this study was subjected to a year of frozen storage. Although freezing can increase the long-term shelf life of a meat, there is the perception that freezing can reduce meat quality (Lagerstedt et al., 2008). Freezing can affect the quality of meat through structural changes that occur due to the formation of ice crystals (Muela et al., 2010). For example, a conventional freezing (-20°C) leads to the formation of irregular, and relatively large, ice crystals (Zhu et al., 2004) which increases the structural damage to the meat (Devine et al., 1995). Therefore, when meat is thawed, these ice crystals can cause physical damage to the microstructures in meat (Muela et al., 2010), reducing the meats ability to retain water.

Some researchers have shown that freezing and thawing results in a product with significantly larger cook losses. Boles and Swan (2002c) reported that frozen storage

time significantly affected the cook yield of beef inside rounds, with the highest yield occurring after 5 weeks of frozen storage. Likewise, Smith et al. (1973) noted that cook losses increased significantly as a result of freezing at -34°C, but not at -23°C. However, Boles and Swan (2002b) reported that freezing and thawing inside rounds before their use in roast beef manufacture both avoided any detrimental effects on processing characteristics while significantly increasing the cook yields of roasts. It should be noted that discrepancies in past studies can be attributed to variations in freezing rates, thawing rates, storage temperatures, and frozen storage duration (Pietrasik and Janz, 2009), while no papers were found that evaluated the quality of beef following one year of frozen storage.

4.4.5 Lipid Oxidation in Pre-Cooked SM Roasts

Similar to the results obtained with the raw injected SM roasts, cooking the SM roast and storing them for an extended period of time did not influence oxidative stability **(Table 4-5)**, although the TBARS values from the pre-cooked roasts prepared from the SM of steers fed a DDGS diet were numerically higher than the TBARS values from the pre-cooked roasts prepared from the SM of steers fed the control diet. Not only was diet insignificant, but day was also insignificant ($P<0.1$), meaning that TBARS had not increased by 56 days of storage. These findings are in accordance with Nuñez de Gonzalez et al. (2009), who observed slight TBARS fluctuations over ten weeks of refrigerated vacuum-packaged storage of sliced hams, with no clear patterns being observed in TBARS values during storage. This is likely due to the absence of air due to vacuum packaging, preventing the unsaturated fatty acids from reacting with reactive oxygen species to form free radicals.

Table 4-5 TBARS values of injected pre-cooked *semimembranosus* roasts obtained from steers fed various DDGS diets.

	Treatment				SEM	Day			SEM*	*P*-value		
		DDGS Diet										
	Control	Corn	Wheat	Blend		1	28	56		Diet	Day	Diet x Day
TBARS[1]												
Injected	1.36	1.63	1.91	1.75	0.198	1.79	1.81	1.39	0.172	0.254	0.166	0.984

* Pooled SEM

[1] mg malondialdehyde per kilogram of beef

Although a day effect was not detected, numerically it can be seen that overall TBARS values decreased from day 28 to day 56. The reason for this is unclear; however, it is possible that the decreased TBARS values could be caused by a decrease in overall malondialdehyde as it was converted to other tertiary oxidation products. In the TBARS experiment the reagent, thiobarbituric acid, measures overall malondialdehyde concentrations (Lynch and Faustman, 2000). However, malondialdehyde is a secondary lipid oxidation breakdown product and, as storage time increases, the malondialdehyde concentration reaches a peak and begins to decrease due to malondialdehyde undergoing further reduction reactions (Gray and Monohan, 1992). Therefore it is possible that the overall reduction in TBARS values is due to the overall decrease in malondialdehyde as a result of this breakdown.

Other research has identified beef with TBARS values of 2.0 or greater as being rancid (Campo et al., 2006). In the present study, TBARS values are well below the limiting threshold for the acceptability of oxidized beef. Thus the combination of brine injection with vacuum-packaging is sufficient to maintain beef quality over 56 d of refrigerated vacuum-packaged storage. These findings agree with those of Robbins et al. (2002), who observed positive effects of phosphates in the moisture enhancement solution on the oxidative stability of injected beef.

4.4.6 Shear Force

Brine injection resulted in a 50% reduction in overall WBSF values compared to non-treated steaks (**Table 4-6**; statistics not shown). The significant improvement in overall shear force values of SM following injection with a salt/phosphate brine is in agreement with results reported by many others (Pietrasik et al., 2005; Pietrasik and

Shand, 2004; Pietrasik and Shand, 2005; Boles and Shand, 2001; McGee et al., 2003). Not only was there myofibrillar swelling initiated by an increased pH as a result of phosphate injection, but salt and phosphate may have acted synergistically to depolymerize myosin filaments and partially dissociate actomyosin (Offer and Trinick, 1983), resulting in a breach of structural integrity and an overall decrease in shear force values (Pietrasik and Shand, 2011). In addition to a breach in structural integrity, an increased number of water-binding sites are liberated as myofibril proteins are extracted from the tissue structure and interact with water via hydrogen bonds, resulting in the formation of a protein-matrix that requires less force to shear (Pietrasik and Shand, 2011).

In addition to salt/phosphate addition, numerous studies have shown that post rigor muscles undergo changes in tenderness during freezing and frozen storage. For example, Hiner et al. (1945) stated that freezing improved the tenderness in unaged beef and hypothesized that this improved tenderness was a result of physical disruption of the muscle cells caused by intracellular ice crystal formation. Intracellular ice crystals formed during fast freezing have been shown to result in fibre splitting and damage which, in some cases, has been associated with greater tenderness (Hiner et al., 1945). Likewise, Smith et al. (1973) reported no effect of freezing on tenderness in beef that was placed in frozen storage for 3-6 weeks but concluded that freezing resulted in a decrease in WBSF values of beef placed in frozen storage for 4 months.

Table 4-6 Effect of diet on shear and expressible moisture of non-injected and injected pre-cooked *semimembranosus* roasts obtained from steers (n=48) fed various DDGS diets.

	Treatment				SEM	Day			SEM*	*P*-value		
		DDGS Diet										
	Control	Corn	Wheat	Blend		1	28	56		Diet	Day	Diet x Day
Non-Injected												
WBSF[1]	92.42	99.93	90.79	99.62	4.43	n/a	n/a	n/a	n/a	0.336	n/a	n/a
SF M	92.20	99.93	90.73	99.61	4.43	n/a	n/a	n/a	n/a	0.324	n/a	n/a
SF C	49.87	50.02	48.93	50.04	3.71	n/a	n/a	n/a	n/a	0.994	n/a	n/a
EM	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Injected												
WBSF[1]	42.31^{ab}	45.55^{a}	41.23^{ab}	40.64^{b}	1.33	43.08	41.08	43.13	1.16	0.037	0.267	0.886
SF M	41.73^{ab}	45.22^{a}	40.58^{ab}	40.23^{b}	1.35	42.61	40.55	42.66	1.18	0.028	0.244	0.892
SF C	31.84	30.99	31.99	31.08	1.01	31.56	31.25	31.61	0.936	0.856	0.949	0.985
EM[2]	13.27^{a}	11.92^{b}	12.84^{ab}	13.64^{a}	0.555	14.22	12.25	12.20	0.530	0.003	<0.001	0.778

* Pooled SEM

n/a = not applicable

[1] WBSF, shear force value (N = newton, SI unit of force), a newton can be converted into kilogram force by dividing the *N* value by 9.80665.

[2] Expressible Moisture, was measured on injected pre-cooked roasts only.

From a dietary standpoint, no tenderness differences were detected in the non-injected SM roasts, which is in agreement with findings of other authors (Gill et al., 2008; Depenbusch et al., 2009; Aldai et al., 2010b) working with the *longissimus* muscle of cattle fed various DDGS diets. Although there were large numerical differences in the non-injected WBSF and myofibrillar tenderness values, there was too much variability, as indicated by the large SEM, for significance to be detected. However, injection with a salt/phosphate brine lowered the variability, resulting in dietary differences in WBSF and myofibrillar tenderness. Specifically, pre-cooked beef roasts prepared from the SM of steers fed corn DDGS were less tender (higher shear values; $P>0.05$) than pre-cooked beef roasts prepared from the SM of steers fed blend DDGS, with pre-cooked roasts prepared from the SM of steers fed wheat DDGS and control displaying intermediate values.

It is not clear why differences in tenderness were observed because there is no difference in overall moisture content or purge loss; however, meat obtained from steers fed corn DDGS had lower expressible moisture values. As previously mentioned, differences in quality have been observed between the deep and superficial SM portions as a result of variations in the rate of pH and temperature decline (Kim et al., 2010). Because the deep SM has a slower chill rate and more rapid pH decline than the superficial SM, protein functionality and water-holding capacity are negatively affected (Sammel et al., 2002). Consequently, this increased protein denaturation can be linked to poor water-holding capacity and perhaps decreased tenderness (Sammel et al., 2002). Because corn DDGS has more energy than barley and wheat DDGS, it is possible that the carcasses of steers fed corn DDGS had more carcass fat insulating the deep SM, preventing the deep SM from chilling properly, resulting in more protein denaturation when compared to the SM of steers fed the other diets. Because of this increased protein denaturation, there is less water available in the meat, as shown by EM, resulting in less tender meat. However, there is not enough evidence to support this theory.

It is also possible that dietary differences were observed as a result of increased protein oxidation in the pre-cooked roasts prepared from the SM of steers fed corn DDGS. Protein oxidation is a topic of increasing interest among meat researchers, with the formation of protein carbonyls from amino acid side chains impairing the

conformation of myofibrillar proteins, leading to denaturation and loss of functionality (Estevez, 2011). Zakrys et al. (2009) confirmed the link between protein oxidation and decreased tenderness in beef muscles as a result of decreased proteolytic degradation of meat proteins during aging and by inducing protein cross-linking via disulphide bonding.

As a result of oxidative stresses in the presence of metal ions, protein carbonyls would be generated from basic amino acids, such as lysine and arginine (Estevez et al., 2011). The loss of protonable amino groups as a result of protein carbonylation would lead to an alteration of the distribution of the electrical charges, impairing the electrical arrangement of meat proteins and modifying the isoelectric point (Estevez et al., 2011). As a result of these chemical modifications, the balance between protein intramolecular interactions and protein-water interactions would be altered, causing a loss of protein solubility while increasing protein-protein interactions, leading to protein denaturation (Estevez et al., 2011). This would cause the myofibrils to shrink, with mechanical constriction preventing swelling (Liu et al., 2009), and, as a result, significantly impact the water-holding capacity of the meat (Estevez et al., 2011). Such an event can explain the lower EM values and higher shear values observed in the pre-cooked roasts prepared from SM obtained from steers fed corn DDGS. Because protein carbonyls were not quantified, there is no concrete data to support this theory; therefore, it is unclear why feeding steers corn DDGS decreased EM and increased shear values of pre-cooked roasts when compared to steers fed blend DDGS.

4.5 Summary

In summary, no dietary differences were observed for meat quality, processing attributes, or tenderness of the non-injected SM roasts. Diet did have an effect on the tenderness of the injected SM roasts, with pre-cooked roasts prepared from the SM of steers fed corn DDGS having higher WBSF and myofibrillar shear values than pre-cooked roasts prepared from the SM of steers fed blend DDGS. This is most likely due to the roasts prepared from the SM of steers fed corn DDGS having less available moisture, as was shown with expressible moisture, than roasts prepared from the SM of steers fed blend DDGS and control.

TBARS analysis of the raw non-injected SM showed that meat obtained from steers fed a DDGS diet was less oxidatively stable than meat obtained from steers fed the control diet. Diet did not have an effect on L* and b* colour values; however, it was observed that steaks from animals fed a DDGS diet lost redness faster over time (lower a* values) than control steaks. These effects were likely caused by the meat from steers fed a DDGS diet having higher levels of total unsaturated fatty acids than the meat obtained from steers fed the control diet. Not only were these dietary effects mitigated with the injection of a salt/phosphate brine, but overall TBARS values were lowered due to the antioxidant effect of phosphates. Likewise, cooking and subsequent refrigerated vacuum-packaged storage maintained the oxidative stability of the pre-cooked SM roasts below levels where rancidity is normally detected over a period of 56 d.

Overall, feeding 40% wheat DDGS, 40% corn DDGS or 40% blend DDGS did not significantly affect meat quality, processing characteristics or oxidative stability of enhanced roasts. Likewise, feeding wheat or blend DDGS did not affect any tenderness parameters; however, feeding corn DDGS resulted in meat with the highest overall WBSF values.

5.0 GENERAL DISCUSSION

The overall objective of the research leading to this thesis was to examine the effect of feeding cattle DDGS derived from wheat, corn or their combination on the resulting meat quality. To accomplish this, 80 crossbred beef steers (20 per diet) were finished on diets containing barley (control) or substitution of barley for either wheat, corn, or wheat/corn DDGS (40% DM basis). These animals were subsequently slaughtered, with the *longissimus* (7^{th} to 12^{th} ribs) and the left and right *semimembranosus* muscles collected for meat quality analysis. Following aging (14d), the *longissimus* was cut into individual steaks for subsequent analysis, while the denuded SM were placed in frozen storage for one year.

In the first phase of the study (Chapter III), individual steaks were removed from the *longissimus* muscle to determine if diet had an effect on meat composition, drip and cook loss, cook time, tenderness, fatty acid composition, oxidative and colour stability, α-tocopherol content, and sensory characteristics. Steaks of predetermined thicknesses were removed from the loin, sealed in an anaerobic environment, and placed in frozen storage until they were analysed. This phase of the study was driven by the premise that "feeding DDGS will improve the healthfulness of the meat, through an improved fatty acid profile, but this improved fatty acid profile will negatively impact the colour and oxidative stability of the meat." The uniqueness of the current research was the inclusion of the blend DDGS diet to assess the impact of feeding a DDGS diet derived from the fermentation of wheat and corn on meat quality. To our knowledge, no research has evaluated the effect of feeding DDGS derived from wheat and corn on resulting meat quality. In addition, no research has been conducted evaluating the impact of feeding wheat DDGS on the oxidative stability of the meat. Similarly, no research has been conducted to examine the impact of DDGS diet on the fatty acid profile of intramuscular fat. Therefore, this research was undertaken to expand on previous research reported by Aldai et al. (2010a; 2010b).

Individual animals were considered the experimental unit throughout this thesis and grouped according to diet. Agreeing with work conducted by Aldai et al. (2010b) who fed similar diets, no dietary differences were reported for chemical composition, cooking parameters, tenderness parameters, or sensory characteristics. In contrast, dietary differences were observed in the fatty acid profiles of both subcutaneous and intramuscular fat. More specifically, feeding a DDGS diet resulted in elevated levels of polyunsaturated fatty acids and conjugated linoleic acid in both the subcutaneous and intramuscular fat. The majority of these findings are in agreement with Aldai et al. (2010a); however, differences in the fatty acid profiles between subcutaneous and intramuscular fat were also identified. In general, the intramuscular fat had lower levels of branched-chain fatty acids, saturated fatty acids and monounsaturated fatty acids than the subcutaneous fat. The lower levels of these fatty acids in the intramuscular fat is most likely related to a dilution by polyunsaturated fatty acids caused by a higher concentration of polyunsaturated fatty acids being present in the intramuscular fat due to the presence of a greater amount of phospholipid membranes.

Although the dilution effect observed in the intramuscular fat can lead to healthier beef, due to the lowering of overall saturated and *trans*-monounsaturated fatty acids, an increase in total polyunsaturated fatty acids can negatively affect colour and oxidative stability of the meat. This occurs because the degree of lipid oxidation is dependent on the degree of polyunsaturation in the fatty acid profile (Chaijan, 2008). Lipid oxidation is one of the main factors limiting the quality and acceptability of meats and other muscle foods (Chaijan et al., 2008) and has long been recognized as a leading cause of quality deterioration in muscle foods, often being the decisive factor in determining food product storage life (Ross and Smith, 2006). This process leads to discolouration, drip losses, off-odour and off-flavour development, texture defects, and the production of potentially toxic compounds (Richards et al., 2002). Because the subcutaneous fat was removed from the loin prior to cutting, only the intramuscular fat was present to impact colour and oxidative parameters.

In the case of other retail meats that contain both subcutaneous and intramuscular fat, such as ground beef, the lower unsaturated fatty acid concentrations in the subcutaneous fat should help lower the meat's susceptibility to colour deterioration and

oxidation. The presence of subcutaneous fat in ground beef should also help to improve the healthfulness of the beef by lowering the n-6:n-3 ratio, bringing it down towards the recommended ratio of 5:1 (WHO, 2003).

Fatty acid analysis indicated that the intramuscular fat of steers fed a DDGS diet contained significantly more polyunsaturated fatty acids than the intramuscular fat of steers fed the control diet. Because of this higher degree of polyunsaturation, it can be expected that meat obtained from steers fed a DDGS diet will be more susceptible to discolouration, oxidation, and the development of off-flavours in the meat. Although it was expected that meat from steers fed a DDGS diet should have some associated off-flavours, no differences in sensory parameters were identified by a trained sensory panel. During loin cutting, the steaks were immediately vacuum-packaged and, following aging, were frozen until it was time for cooking. Because the steaks used for sensory analysis weren't exposed to conditions favouring the onset of lipid oxidation for prolonged periods of time, off-flavours commonly associated with lipid oxidation possibly did not develop in the meat. Therefore, further research is needed to determine if storing meat from steers fed a DDGS diet under retail conditions impacts the meat's overall flavour profile, especially in regards to meat stored under high oxygen conditions.

Differences were detected in the colour stability of meat obtained from steers fed a DDGS diet versus steers fed the control. Specifically, meat obtained from steers fed a DDGS diet lost redness (a* value) at a faster rate and thus were less colour stable than meat obtained from steers fed the control diet. These findings were expected due to the higher levels of polyunsaturated fatty acids in the intramuscular fat and were subsequently reinforced through subjective colour analysis, where it was observed that steaks obtained from steers fed the control diet had a more desirable retail appearance than steaks obtained from steers fed blend DDGS.

As was observed with meat colour, it should be expected that meat obtained from steers fed a DDGS diet should be less oxidatively stable, and thus would display higher TBARS values than meat obtained from steers fed the barley control diet. However, differences were not observed in TBARS values for raw or cooked ground beef. The reason for these findings is unclear, considering the fact that the intramuscular fat of steers fed a DDGS diet contained significantly higher levels of polyunsaturated fatty

acids compared to the control. Consequently, this effect may have been explained by elevated levels of α-tocopherol in the intramuscular fat of steers fed a DDGS diet; however, the findings in this study (no differences in α-tocopherol) do not support this theory.

The significance of the first phase of the study was that feeding beef steers DDGS derived from wheat and/or corn improved the healthfulness of the beef, but the cost was poor colour stability in the *longissimus* muscle. However, the poor colour stability of steaks obtained from steers fed a DDGS diet should not be a major concern to retailers because differences were only detected after the fourth day of retail display, which is typically the longest amount of time retailers will leave meat on display.

Completion of this first phase of the study did raise some questions, which led to the design of the second phase of the study. In particular, there was interest in determining the effect of DDGS diet on the quality of other primal cuts and how this affects the quality of value-added meat products. To accomplish this task, a subset of left *semimembranosus* muscles were removed from frozen storage (stored > 12 months) and thawed, with half of the *semimembranosus* muscle being used for raw meat analysis and the other half undergoing injection with a salt/phosphate brine for analysis as a pre-cooked SM roast. Although differences in TBARS values were not detected in the *longissimus* muscle despite there being differences in fatty acid composition, this research was conducted under the premise that feeding steers a DDGS diet will negatively impact the oxidative stability of meat; therefore, the major goal of the second phase of the study was to determine if there were differences in the oxidative stability of the *semimembranosus* muscles, especially after frozen storage, and to determine if further processing mitigated those effects or enhanced them. However, attention was also paid to examining the colour stability of raw SM steaks and to determine if differences arose in processing parameters, tenderness, or water-holding capacity over a 56 d storage period for pre-cooked SM roasts.

The uniqueness of this study lay in the use of SM's to prepare value-added pre-cooked beef roasts. To our knowledge, no research has been conducted examining the effect of feeding steers a DDGS diet on the quality of muscles other than from the *longissimus.* Furthermore, no research has been conducted evaluating the effect of

feeding finishing steers a DDGS diet on the quality of value-added beef products. Therefore, by preparing pre-cooked beef roasts out of a portion of the SM and storing the product under dark, anaerobic conditions for 56 days, knowledge can be gained on how DDGS diet will impact the quality of an oxidatively stressed value-added meat product as it moves through the distribution channels and into the retail store.

Unlike the first portion of this study which evaluated the overall quality of the *longissimus* muscle, this portion of the study was separated into a section examining raw meat quality and a section examining pre-cooked roast quality. Unlike what was observed in raw ground beef obtained from the *longissimus* muscle, a dietary effect was observed in raw ground beef obtained from the SM. Specifically, raw ground beef obtained from steers fed a DDGS diet displayed significantly higher TBARS values than raw ground beef obtained from steers fed the control diet. This effect was expected due to the higher levels of polyunsaturated fatty acids being present in meat obtained from steers fed a DDGS diet but was not detected in the *longissimus* muscle, possibly due to the *longissimus* muscle being stored for a shorter period of time.

Similar to the first portion of the study, the colour stability of raw SM steaks was also examined in a retail display setting. The *semimembranosus* muscle underwent rapid colour deterioration, taking only 4 d to reach an average a* value of 14 compared to the *longissimus* muscle, which took an average of 7d to reach the same a* value. There may be several reasons to explain why *semimembranosus* colour deteriorated at a faster rate than *longissimus* muscle analysed in the first study. Firstly, fresh meat was used for colour analysis of the *longissimus* muscle, while previously frozen meat was used for colour analysis of the *semimembranosus* muscle. Freezing meat can have an impact on colour stability because freezing and thawing has been reported to have adverse effects on the surface colour of beef (Lanari et al., 1993). For example, Boles and Swan (2002b) reported a decrease in redness after freezing *semimembranosus* muscles from Brahman cattle. Secondly, the *longissimus* muscle is more colour stable than the *semimembranosus* muscle. This is supported by Dunne et al. (2005), who, using a* value, observed that the *longissimus* muscle was more colour stable than the *semimembranosus* muscle.

Shear value results also displayed some interesting trends between the two muscles. When examining the effect of DDGS diet on overall shear values, no dietary

differences were detected for the *longissimus* or *semimembranosus* muscles; however, meat obtained from steers fed corn and blend DDGS displayed numerically higher shear force values than meat obtained from steers fed the control or wheat DDGS. Once the *semimembranosus* muscles underwent injection, a dietary effect was detected in shear values. The reason for this is unclear but it is possible that the overall lowering of shear values due to brine inclusion helped to decrease animal variation, as was shown by the lowering of the SEM, allowing the statistical model to detect significance.

6.0 GENERAL CONCLUSIONS

Feeding steers a finishing diet consisting of DDGS derived from wheat and/or corn resulted in some quality changes in the *longissimus* and *semimembranosus* muscles. Although differences were not detected in meat composition, tenderness, or sensory characteristics of the *longissimus* muscle, some differences were detected in the fatty acid composition of the subcutaneous and intramuscular fat. It was observed that fat obtained from steers fed a DDGS diet contained elevated levels of polyunsaturated fatty acids. As a result, meat obtained from the *longissimus* muscle of steers fed a DDGS diet underwent discolouration at a faster rate than meat obtained from steers fed the control diet.

Similar to what was observed in the *longissimus* muscle, dietary differences were not detected in terms of meat quality attributes or processing parameters for the *semimembranosus* muscle; however, differences were detected in colour and oxidative stability. As in the first study, meat obtained from steers fed a DDGS diet underwent discolouration at a faster rate and displayed higher TBARS values than meat obtained from steers fed the control diet. Although inclusion of salt/phosphate brine lessened the effect of diet on oxidative stability by lowering overall TBARS values, there was still a trend for injected ground beef obtained from steers fed a DDGS diet to display higher TBARS values.

Injection with salt and phosphate did emphasize some effects that were not detected in the non-injected *longissimus* and *semimembranosus* muscles. Specifically, injected SM obtained from steers fed corn DDGS had elevated shear values when compared to injected SM obtained from steers fed the other diets. Overall, although some minor effects were detected, no major differences existed in meat obtained from steers fed different DDGS diets. Therefore, industry should continue the practice of feeding DDGS to feedlot cattle on the premise that feeding a DDGS diet improves the healthfulness of the meat with little impact on meat quality or processing attributes. However, it should be cautioned that meat obtained from cattle fed a DDGS diet should

be properly stored to avoid quality problems associated with meat products containing elevated levels of polyunsaturated fatty acids, such as more rapid colour deterioration or the onset of lipid oxidation, which can result in the formation of off-flavours and off-odours.

Feeding a DDGS diet results in elevated levels of polyunsaturated fatty acids in both the subcutaneous and intramuscular fat, hence further research can be conducted to evaluate the impact of these higher levels in other meat applications. For example, ground beef contains both intramuscular and subcutaneous fat; therefore, research could be conducted to evaluate the impact of combining these two fat sources on colour deterioration, oxidative stability and sensory characteristics. Furthermore, future studies could evaluate the impact of storing meat obtained from steers fed a DDGS diet under normal distribution and retail display conditions on eating quality to determine if the onset of lipid oxidation impacts sensory characteristics.

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